

# UC Santa Barbara

## UC Santa Barbara Previously Published Works

### Title

Evaluation of frameworks proposed as protective of antimicrobial resistance propagation in the environment

### Permalink

<https://escholarship.org/uc/item/6kg3q4gj>

### Authors

Mortimer, Monika  
Winchell, Alyssa  
Holden, Patricia A

### Publication Date

2020-11-01

### DOI

10.1016/j.envint.2020.106053

Peer reviewed



# Evaluation of frameworks proposed as protective of antimicrobial resistance propagation in the environment



Monika Mortimer<sup>a,b,\*</sup>, Alyssa Winchell<sup>b</sup>, Patricia A. Holden<sup>b,\*</sup>

<sup>a</sup> Institute of Environmental and Health Sciences, College of Quality and Safety Engineering, China Jiliang University, Hangzhou, Zhejiang 310018, China

<sup>b</sup> Bren School of Environmental Science and Management and Earth Research Institute, University of California Santa Barbara, Santa Barbara, CA 93106, United States

## ARTICLE INFO

Handling editor: Olga Kalantzi

### Keywords:

Antimicrobial resistance  
Surface waters  
PNEC  
Antibiotic concentrations  
Environment  
Human health

## ABSTRACT

Antimicrobial resistance (AMR) in the environment is a globally concerning issue. This study sought to improve the understanding of human health risks from an environmental AMR proliferation perspective. Surface water concentrations of 11 most used antibiotics in the United States were simulated for the Columbia and Sacramento River watersheds using the Pharmaceutical Assessment and Transport Evaluation (PhATE) model. The predicted environmental concentrations (PECs) and literature-reported measured environmental concentrations (MECs) of antibiotics were compared to the predicted no effect concentrations (PNECs) of three frameworks proposed as protective of AMR selection. For all of the studied antibiotics, PECs (except for moxifloxacin, a 4th generation fluoroquinolone), and at least one published MEC, were above the safe limit proposed by at least one of the three frameworks. The results indicate that a variety of different antibiotics with different mechanisms of action and physico-chemical properties are likely in environmental compartments at or above the concentrations currently proposed as safe from an AMR proliferation perspective. Understanding environmental occurrence of antibiotics is important for assessing environmental exposures and, when compared to PNECs for resistance selection, can—either alone or in combination with other methods— more specifically indicate where there are potential risks of AMR proliferation.

## 1. Introduction

Subtherapeutic levels of antibiotics in the aquatic environment pose a potential threat to both human health and the integrity of natural ecosystems. Antibiotic pollution has been implicated in the exacerbation of antimicrobial resistance (AMR; [Rodríguez-Mozaz et al., 2015](#)) and in influencing non-target organisms, putting at risk the function of organisms that perform essential services in the environment ([Grenni et al., 2018](#)). In addition to exerting selective pressure on environmental microbiomes ([Xiong et al., 2015](#)), the release of antibiotic residues in the environment may also pose hazard to human microbiomes via ingested food or drinking water ([Ben et al., 2019](#)). However, current risk assessment practices are inadequate to evaluate the effect of antibiotics on AMR emergence and selection, especially in non-clinical environments ([Vikesland et al., 2017](#)).

Based on current regulations, the potential environmental risk of pharmaceutical substances, like all other chemicals, is calculated from the ratio between the predicted environmental concentration (PEC) of the substance in the aquatic environment and the predicted no effect

concentration (PNEC), a concentration below which no adverse effects on the environment are expected ([Lee and Choi, 2019](#)). Among the countries where assessment of environmental impacts of human pharmaceuticals before marketing authorization is required by law — the EU ([Directive, 2001/83/EC](#)), the U.S. (National Environmental Policy Act of 1969), and Canada (Canadian Environmental Protection Act of 1999) — the EU has recently adopted a tailored risk assessment for antibiotics ([EMA, 2018](#)). Despite the antibiotic-specific assessment requirements, these may not be followed in cases where the released amounts of antibiotics are predicted to be low. These regulations overlook the aspect that antibiotic effects can have broader impacts than causing toxicity. For instance, sub-minimum inhibitory concentrations (sub-MICs) of antibiotics may select for AMR ([Gullberg et al., 2011](#); [Andersson and Hughes, 2012](#)). The emergence and mobilization of novel resistance genes in environmental bacteria and subsequent transfer to human pathogens has been identified as a major human health risk associated with environmental AMR ([Bengtsson-Palme et al., 2018](#)). These events are more likely to occur in environments under strong selection pressures such as these exerted by

\* Corresponding authors at: Room 308-2, Fangzheng Building, China Jiliang University, 168 Xueyuan Street, Hangzhou, Zhejiang 310018, China (M. Mortimer). 3508 Bren Hall, Bren School of Environmental Science & Management, University of California, Santa Barbara, CA 93106-5131, USA (P.A. Holden).

E-mail addresses: [mmortimer@cjlj.edu.cn](mailto:mmortimer@cjlj.edu.cn) (M. Mortimer), [awinchell@bren.ucsb.edu](mailto:awinchell@bren.ucsb.edu) (A. Winchell), [holden@bren.ucsb.edu](mailto:holden@bren.ucsb.edu) (P.A. Holden).

<https://doi.org/10.1016/j.envint.2020.106053>

Received 20 April 2020; Received in revised form 20 July 2020; Accepted 9 August 2020

0160-4120/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

antibiotic pollution from industrial sources. Moreover, the effects of antibiotics may be potentiated by other stress factors or contaminants, such as heavy metals and biocides, which may enhance the spread and evolution of AMR (Davies, 2009; Seiler and Berendonk, 2012). While not all contributing factors and routes of transfer of resistance genes from environmental bacteria to human pathogens are understood, one of the interventions that can be taken for mitigation of environmental AMR selection involves better control of antibiotic discharges from manufacturing plants (Berendonk et al., 2015; Bengtsson-Palme et al., 2018).

Considering the uncertainties involved in correlating environmental antibiotic concentrations with AMR proliferation, it is difficult to define safe release levels in a strict sense. However, the scientific community and industry stakeholders have made efforts to estimate antibiotic concentrations that, based on current empirical knowledge, should provide safety limits for protecting human health from risks of AMR selection (Bengtsson-Palme and Larsson, 2016; Le Page et al., 2017; Tell et al., 2019). Specifically, two international frameworks have been proposed which have established PNECs protective of AMR selection: i) Bengtsson-Palme and Larsson (2016) proposed the establishment of compound-specific safe antibiotic emission limits (PNECs) derived from MICs for clinically relevant bacteria; and ii) Le Page et al. (2017) suggested a single-value production discharge limit of 100 ng/L, based on no observed effect concentrations (NOECs) of antibiotics for environmental bacteria and minimum selective concentrations (MSCs) for clinical bacteria, to be protective of ecosystems and AMR development. The latter framework was later updated with antibiotic-specific PNECs based on the ecotoxicology data generated by a group of pharmaceutical companies (AMR Industry Alliance) as per Brandt et al. (2015).

To test the ecological relevance of their proposed PNECs, Bengtsson-Palme and Larsson (2016) compared measured antibiotic concentrations in municipal sewage treatment plant effluents to PNECs and found that, in the case of 28% of antibiotics, the effluent concentrations exceeded the PNECs. These results indicated that advanced treatment may be needed for reduction of AMR selection in treatment plants. Still, post-discharge environmental fate and transport processes, including dilution and degradation, may influence effluent antibiotics' concentration effects on receiving water bodies at the watershed scale. However, the proposed PNECs have not been evaluated with regard to PECs or measured environmental concentrations (MECs) of antibiotics in surface waters. Further, simultaneous co-consideration of current international frameworks for guiding antibiotic PNECs for reducing AMR proliferation and human health risk has not been performed. Here, we asked: for antibiotics in major use in the U.S., how do PECs and/or MECs compare at the watershed scale to PNECs, according to current international frameworks?

Accordingly, the aim of this study was to evaluate the performance of the proposed frameworks (Bengtsson-Palme and Larsson, 2016; Le Page et al., 2017) in the context of environmental antibiotic concentrations in surface waters for two major U.S. watersheds and compare the performance of published frameworks to in-house calculated highest acceptable human drinking water concentrations (HDWC) of antibiotics, protective of AMR. For this, PECs in the Columbia and Sacramento watersheds were simulated for the 11 most sold antibiotics in the U.S. (FDA, 2012; Table 1) and compared to PNECs for AMR selection: 1) proposed by Bengtsson-Palme and Larsson (2016); 2) proposed by Le Page et al. (2017) and updated by AMR Industry Alliance (Tell et al., 2019) and 3) the HDWC of antibiotics, based on the guidance issued by Food and Drug Administration's Center for Veterinary Medicine (VICH, 2012). PNECs and modeled PECs were also compared to literature-reported MECs of antibiotics for surface waters. The study aims to place the established safe antibiotic threshold concentrations (PNECs), intended for contributing to the reduction of AMR emergence and selection in the environment, in the context of predicted antibiotic levels in two North American rivers and globally reported surface water antibiotic MECs. The presented analysis contributes to the

**Table 1**  
Antibiotics, their classification and national sales.

| Antibiotic       | Antibiotic sub-class        | National sales (kg/year) <sup>1</sup> |
|------------------|-----------------------------|---------------------------------------|
| amoxicillin      | Penicillins                 | 1,140,920                             |
| cephalexin       | Cephalosporins (1st gen.)   | 298,205                               |
| ciprofloxacin    | Fluoroquinolones (2nd gen.) | 209,832                               |
| levofloxacin     | Fluoroquinolones (3rd gen.) | 55,827                                |
| moxifloxacin     | Fluoroquinolones (4th gen.) | 11,003                                |
| doxycycline      | Tetracyclines               | 64,956                                |
| clindamycin      | Lincosamides                | 71,173                                |
| azithromycin     | Macrolides                  | 104,499                               |
| metronidazole    | Nitroimidazoles             | 120,021                               |
| sulfamethoxazole | Sulfonamides                | 398,379                               |
| trimethoprim     | Microbial DHFR inhibitors   | 81,304                                |

DHFR - dihydrofolate reductase

<sup>1</sup> Antibiotics sold in 2011 by manufacturers across all combined retail (chain, independent, food store, mail order, discount house, and mass merchandise pharmacies) and non-retail (hospitals, long-term care facilities, clinics, home healthcare providers, and health maintenance organizations or HMOs) U.S. distribution channels (FDA, 2012).

understanding of human health risks associated with environmental AMR.

## 2. Materials and methods

### 2.1. Determination of PECs of antibiotics using the PhATE model

Antibiotic concentrations in Columbia and Sacramento watersheds were predicted using the PhATE (Pharmaceutical Assessment and Transport Evaluation) model (Anderson et al., 2004). The model was developed as a tool to estimate concentrations of active pharmaceutical ingredients (APIs) in the United States surface waters that result from patient use (or consumption) of pharmaceuticals. The model divides rivers into discrete segments. It estimates the mass of API that enters a segment from upstream or from the wastewater treatment plant (WWTP), and is subsequently lost from the segment via in-stream loss mechanisms or flow diversions (i.e., manmade withdrawals). WWTP discharge loads are estimated based on the population served, the API use per capita, the potential loss of the compound associated with human use (e.g., metabolism), and the portion of the API removed in the WWTP (Table 2).

#### 2.1.1. Literature search

The values for antibiotic physico-chemical properties (water solubility, biodegradation rate coefficients, pK<sub>a</sub>, n-octanol/water partition coefficient or logK<sub>ow</sub> and chemical type; Table S1) and input values in PhATE to account for WWTP and in stream losses of antibiotics were collected from peer reviewed sources that were retrieved from EBSCOhost databases (GreenFile, Academic Search Complete, Environment Index), SciFinder, and Google Scholar between July 2018 and September 2018.

#### 2.1.2. Determination of antibiotic regional usage rates for Sacramento and Columbia watersheds

National- and state-level antibiotic usage rates in number of prescriptions per 1,000 people during 2011 were retrieved from the Patient Safety Atlas of the Centers for Disease Control and Prevention (CDC, 2011; Table S2) and were used for calculations of State per capita usage, the input for the PhATE model, as explained in the SI. The dataset contains dispensing data for oral antibiotic prescriptions that are extracted from the Xponent database from QuintilesIMS (Danbury, Connecticut). QuintilesIMS collects dispensing data from community and mail-order pharmacies which report their entire business to QuintilesIMS each week. QuintilesIMS reports capturing > 70% of outpatient prescriptions dispensed in U.S. community and mail-order

**Table 2**  
Summary of key inputs for PhATE model (Anderson et al., 2004).

| Parameter type        | Parameter Category  | Parameter                               | Unit  | Information source   |   |
|-----------------------|---------------------|---|---|--|---|
| User input            | API                 | Usage per capita                        | kg/person-year                              | Centers for Disease Control and Prevention; 2012 FDA Antibiotic Drug Use Review; U.S. Census Bureau (2012) |   |
|                       |                     | In-stream first-order loss coefficients | day <sup>-1</sup>                           | Peer-reviewed literature   |   |
|                       |                     | Loss by human metabolism                | %   | FDA-approved prescribing information   |   |
| Provided in the model | WWTP                | API removal efficiency                  | %   | Peer-reviewed literature   |   |
|                       |                     | Name, location and type                 | NA  | BASINS/U.S. EPA Clean Water Needs Survey-1996 and BASINS/U.S. EPA Permit Compliance System                 |   |
|                       |                     | Population served                       | persons                                     |  |   |
|                       | Dams and reservoirs |   | Flow rate                                   | m <sup>3</sup> /day  |   |
|                       |                     |   | Names                                       | NA   | BASINS/U.S. Army Corps of Engineers National Inventory of Dams          |
|                       |                     |   | Volumes                                     | m <sup>3</sup>   |   |
|                       | River segments      |   | Surface areas                               | m <sup>2</sup>   |   |
|                       |                     |   | Lengths and depths                          | m  |   |
|                       |                     |   | Numbers and sequences                       | NA   | RF1, complemented with USGS Enhanced River Reach File 2.0 (ERF1-2) data |
|                       |                     |   | Mean and low flow (7-day, 10-year low flow) | NA   |   |
|                       |                     |   | Mean and low-flow velocity                  | m <sup>3</sup> /day  |   |
|                       |                     |   | Length, depth and width                     | m  |   |

API - active pharmaceutical ingredient, WWTP – wastewater treatment plant.

pharmacies and reconciles them to wholesale deliveries. Then, using a patented projection methodology, QuintilesIMS projects to 100% coverage of dispensed medications to produce estimated prescription counts. Antibiotic usage rates for each antibiotic were refined by class when available (“state data” for 4 antibiotic classes were available); otherwise data from the “all classes” category were collected (Table S2).

National drug sales statistics for all antibiotics were retrieved from a U.S. Food and Drug Administration (FDA) analysis reporting 2011 masses (kg) of clinical antibiotics sold (Table 1; FDA, 2012). The analysis included selected systemic antibacterial drug products sold from manufacturers to various retail and non-retail channels of distribution as a surrogate for nationwide antibacterial drug use in humans. The PhATE model assumes that per capita usage is the same across the entire U.S. To account for regional variation in antibiotic usage, the sales data were adjusted to the Columbia and Sacramento River watersheds (Tables S3 and S4). In the PhATE model, the Columbia River watershed is contained within Idaho, Montana, Oregon, and Washington, while the Sacramento watershed predominately resides inside of California. The PhATE model uses annual mass (kg per year) as input for drug usage, which it converts to usage per capita by dividing the mass by the total population of the U.S. The antibiotic usage (kg/year) input for each watershed (i.e., Sacramento and Columbia River) was modified so that the per capita values would reflect the State per capita usage (for calculations see SI).

This study used drug consumption data from 2011 because, at the time of the planning and during data collection, the latest antibiotic sales data, sourced from the FDA Antibiotic Drug Use Review, was available for 2011. This, however, is not expected to affect the current impact of the study due to small number of new antibiotics in the development phase, the median of 6-year clinical trial time and 8-month FDA review time (Deak et al., 2016).

### 2.1.3. Accounting for human metabolism of antibiotics

Antibiotic metabolism rate information was obtained from drug information sheets registered with the FDA. The fraction of active ingredient excreted in urine, and fecal matter when available, was used to calculate the inputs for the fractional loss from humans for PhATE by subtracting the fraction excreted from 1 (Table S5). Fractional losses of metronidazole, levofloxacin, and moxifloxacin include excretion both in urine and fecal matter. When more than one value for the fraction of active ingredient excreted was given, the higher value was chosen for a conservative estimate.

### 2.1.4. Accounting for antibiotic removal in WWTPs

All WWTPs in both watersheds provide secondary treatment, “advanced treatment I”, or “advanced treatment II” (PhATE User Manual V4.0). Advanced treatment I is defined in the PhATE user’s manual as “10 ≤ biological oxygen demand (BOD) < 25 mg/L and/or nutrient removal,” while advanced treatment II is described as “BOD < 10 mg/L and/or nutrient removal.” WWTP aqueous removal efficiencies of antibiotics during secondary treatment were found in the literature (Table S5). Search terms during the literature search included either “antibiotic” or the name of a given active ingredient AND “wastewater” AND “review” AND “removal.” When possible, averaged values measured from multiple WWTPs were selected as input for PhATE. For a conservative estimate, the secondary values were also used for advanced treatment I and II. The removal efficiency percentages were entered into PhATE as the WWTP fractional losses. For clindamycin, the only value available in the literature for WWTP aqueous removal efficiency was a negative value (-1.5). This may be caused by very low concentrations (0.002–0.005 ng/L) reported in the influent and effluent and possible measurement errors (Verlicchi et al., 2012). In PhATE, the fractional secondary WWTP loss was set at 0 for clindamycin.

### 2.1.5. Accounting for antibiotic in-stream loss

In-stream loss coefficients for the antibiotics were collected from the literature (Table S5). A literature search was completed with the following search terms: the active ingredient name AND (hydrolysis OR aqueous OR “waste water” OR wastewater) AND (rate OR degradation) AND (effluent), as well as photodegradation, environmental fate, persistence, microcosm, and simulated environment. Values were selected from studies simulating the natural environment through microcosms, simulated natural light, simulated or real stream water, or other similar methods estimating the fate and persistence of antibiotics in the aquatic environment. Values that were reported as half-lives were converted to first order rate loss coefficients by dividing 0.6931 by the half-life. The in-stream loss input for doxycycline was reported in the literature as a percent loss, and was input into PhATE as such (Zaranyika et al., 2015).

## 2.2. Characterization and evaluation of frameworks

In this study, the approach proposed by, and the PNECs derived in, Bengtsson-Palme and Larsson (2016) are referred to as the “Larsson framework”. PNECs in this framework were derived by extrapolating MIC values for clinically relevant bacteria from the EUCAST database and applying a safety factor of 10 to account for AMR selection risk. In the second framework considered here, by Le Page et al. (2017), a safe

limit for antibiotic manufacturing discharges was derived from aquatic ecotoxicity data for antibiotics (NOECs and 50% effective concentrations or EC<sub>50</sub>) and MSCs for clinically relevant bacteria. A single value of 100 ng/L for all antibiotics was proposed to be protective of environmental bacterial populations with 95% confidence and to conform with the lowest empirical data for AMR selection. However, it was also suggested that the value could be used as an interim measure in the absence of reliable empirical clinical, and environmental, data. To update the data, the members of the AMR Industry Alliance conducted ecotoxicological tests with antibiotics and provided antibiotic-specific “environmental PNEC” values (Tell et al., 2019) for the updated “Le Page framework”. The third framework which was evaluated here, along with the two published frameworks, involved HDWCs calculated per the guidance by the FDA’s Center for Veterinary Medicine (VICH, 2012) with modifications which accounted for potential AMR selection in clinically relevant bacteria. The latter framework is referred to as the “VICH framework”. The frameworks were evaluated by calculating the ratios between the antibiotic PEC obtained by the simulations as described above and the PNECs proposed in the Larsson and Le Page frameworks and HDWCs as per the VICH framework. The values for the Larsson framework PNECs originate from Bengtsson-Palme and Larsson (2016) and the Le Page framework PNECs were taken from AMR Industry Alliance report (IFPMA, 2018; Tell et al., 2019) when available. For all antibiotics with no available value from the AMR Industry Alliance, the standard limit of 100 ng/L was used according to the recommendation by Le Page et al. (2017). VICH framework HDWC values were determined as described below.

### 2.3. Calculation of highest acceptable human drinking water concentrations (HDWCs)

According to VICH guidance (VICH, 2012), calculations of safe antibiotic levels in drinking water should consider two aspects: (1) protection against disruption of the intestinal colonization barrier, and (2) protection against the increase of resistant populations of bacteria. Thus, microbiologically acceptable daily intake (ADI) values are recommended to be calculated for each of these considerations separately, and the lower value should be used for estimation of the HDWC.

For calculating the ADI for the intestinal colonization barrier disruption consideration, calculated minimum inhibitory concentrations (MIC<sub>calc</sub>) were determined, using minimum inhibitory concentrations at which growth of 50% of the bacterial isolates is inhibited (MIC<sub>50</sub>) for a set of ten specified genera of human intestinal microflora recommended by the VICH guidance (Table S6). MIC<sub>50</sub> were used rather than MIC<sub>90</sub> because the intent of the microbiological ADI is to protect normal growth of intestinal microflora, not inhibit growth. The lowest MIC<sub>50</sub> values reported in the Antimicrobial Index, <http://antibiotics.toku-e.com/> (Amirkia and Qiubao, 2011) were selected, and values  $\geq 32 \mu\text{g}/\text{mL}$  were considered intrinsically resistant, i.e., excluded from calculations. Since data were not available for all genera, the lowest available MIC<sub>50</sub> values were divided twice by two (4-fold). This approach is assumed to provide sufficient protection for species that may be sensitive to lower antibiotic concentrations than the lowest reported MIC<sub>50</sub>. The data were log-transformed before calculating means and standard deviations.

For calculating the ADI to protect against the population increases of resistant bacteria, calculated no-observable adverse effect concentrations (NOAEC<sub>calc</sub>) were derived from the minimum MIC (MIC<sub>min</sub>) for medically relevant sensitive bacterial genera, i.e., nonresistant genera (Table S7). MIC<sub>min</sub> data were again collected from the Antimicrobial Index. MIC<sub>min</sub> values were divided three times by two (8-fold) and then log-transformed before calculating means and standard deviations. Detailed calculations of MIC<sub>calc</sub>, NOAEC<sub>calc</sub>, and microbiological ADI ( $\mu\text{g}/\text{kg}$ ) (Table S8 and S9) are described in the SI. The microbiological ADI ( $\mu\text{g}/\text{kg}$ ) was used to calculate the HDWC using the principles of U.S. EPA Water Quality Criteria Guidance (EPA, 2000) by

assuming that humans could be exposed to an antibiotic via drinking 2 L of water (for an adult) as well as eating an average of 17.5 g of fish from water near the drinking water intake (see SI for calculations).

## 3. Results

### 3.1. PECs of antibiotics in the Sacramento and Columbia River watersheds

The PhATE model was applied to the Sacramento and Columbia River watersheds, assuming two different river flows (mean and low flow) and four different loss scenarios: 1) “All”, which included in stream loss and WWTP loss; 2) “In Stream”, which included in stream loss only; 3) “WWTP”, which included loss from WWTPs only; and 4) “None”, which was the most conservative assumption with neither WWTP or in stream loss. All four types of loss scenarios considered loss by human metabolism. The model output was antibiotic concentrations in each river segment (750 segments in the Columbia River and 55 segments in the Sacramento River). Concentrations representing the mean, 90th, 95th and 99th percentile in the river segments were calculated (Table A1 and A2). The antibiotic concentrations simulated when assuming “no loss” were expectedly higher compared to the values obtained when assuming in stream and WWTP losses together, i.e., “all loss” conditions. However, the magnitude of difference between concentrations was antibiotic specific. For clindamycin, azithromycin, sulfamethoxazole, trimethoprim and levofloxacin the differences between the mean concentrations under the “all loss” and “no loss” conditions were only between 2 and 6 times, whereas the concentrations of amoxicillin, doxycycline and cephalixin were one order of magnitude higher than the “no loss” modeling assumption. The simulated mean concentrations of ciprofloxacin, metronidazole and moxifloxacin, in contrast, were three orders of magnitude higher at “no loss” settings than when assuming “all loss”.

### 3.2. Comparison of PECs of antibiotics in the Sacramento and Columbia River watersheds to PNECs of Larsson, Le Page and VICH frameworks

PNECs of the Larsson and Le Page framework for the selected antibiotics were sourced from published literature as described in section 2.2. (Table 3). Additionally, HDWCs for selected antibiotics were determined using publicly available MIC<sub>min</sub> values, since no NOAEC values—which are recommended as a point of departure for HDWC calculations—were available. The NOAEC<sub>calc</sub> was derived using assessment factors to take into account that AMR can be induced at 1/230 to 1/4 MICs, depending on the bacterial genus and environmental conditions (Gullberg et al., 2011). The calculated NOAEC<sub>calc</sub> values

**Table 3**

Predicted no effect concentrations (PNECs) of the Larsson and Le Page frameworks and highest acceptable human drinking water concentrations (HDWCs) of the VICH framework.

| Antibiotic       | Larsson <sup>1</sup> (ng/L) | Le Page <sup>2</sup> (ng/L) | VICH <sup>3</sup> (ng/L) |
|------------------|-----------------------------|-----------------------------|--------------------------|
| amoxicillin      | 250                         | 100                         | 552                      |
| cephalexin       | 4000                        | 80 <sup>a</sup>             | 76,350                   |
| ciprofloxacin    | 64                          | 450 <sup>a</sup>            | 208                      |
| levofloxacin     | 250                         | 100                         | 1490                     |
| moxifloxacin     | 125                         | 100                         | 272                      |
| doxycycline      | 2000                        | 100                         | 984                      |
| clindamycin      | 1000                        | 100                         | 290                      |
| azithromycin     | 250                         | 20 <sup>a</sup>             | 10                       |
| metronidazole    | 125                         | 100                         | 2360                     |
| sulfamethoxazole | 16,000                      | 600 <sup>a</sup>            | 1232                     |
| trimethoprim     | 500                         | 100,000 <sup>a</sup>        | 63,214                   |

<sup>1</sup> Bengtsson-Palme and Larsson (2016);

<sup>2</sup> Le Page et al. (2017);

<sup>3</sup> Calculated in this study;

<sup>a</sup> IFPMA (2018); Tell et al. (2019)

were lower than the  $MIC_{calc}$  values that were derived from published  $MIC_{50}$  values (Table S8). Thus, the microbiological ADI values derived from the  $NOAEC_{calc}$  were used in the HDWC calculations for all of the selected antibiotics. The resulting HDWCs were, overall, much lower than previously reported for the same antibiotics using the ADI calculation approach. For example, Schwab et al. (2005), reported PNEC values, which correspond to the HDWCs calculated here (Table 3), for doxycycline 430  $\mu\text{g/L}$ , ciprofloxacin 23  $\mu\text{g/L}$ , sulfamethoxazole 1900  $\mu\text{g/L}$  and trimethoprim 60  $\mu\text{g/L}$  (Schwab et al., 2005). These values are 2–3 orders of magnitude higher than the HDWCs in this study, except for trimethoprim, for which the HDWC was at a comparable concentration. The reason for these differences is the application of more conservative safety factors in this study, to account for AMR development, which was not considered by Schwab et al. (2005). Consequently, the VICH framework limit concentrations in this study are, in general, lower than estimated in previous reports.

### 3.2.1. Comparison of PECs to PNECs by river segments

PEC values simulated using PhATE for each river segment were compared to the PNEC values established in each of the three frameworks (Table 3) and the results were expressed as a fraction of river segments which exceeded the PECs for antibiotics (percent segments above Larsson, Le Page, or VICH limits, Tables A1 and A2). Expectedly, for both the Columbia and Sacramento River watersheds, the fraction of river segments where the limit concentrations were predicted to be exceeded was larger for simulated low flow conditions than for mean flow conditions (Figs. 1 and 2). Overall, the patterns across different “loss scenarios”, frameworks, and antibiotic types were similar for the two rivers (correlation coefficients > 0.9). Under the most conservative assumptions of no antibiotic loss and low flow conditions (Fig. 1D and Fig. 2D), the antibiotics estimated to reach concentrations above all framework limits were amoxicillin (21–49% of segments in Columbia and 33–64% in Sacramento River waters) and ciprofloxacin (10–22% of segments in Columbia and 15–35% in Sacramento River waters).

Levofloxacin, metronidazole, azithromycin and sulfamethoxazole concentrations were predicted to be above at least two frameworks’ limits in at least 10% of river segments both in the Columbia and Sacramento River watersheds. Cephalixin was estimated to be over the Le Page framework limit in 32% of segments in the Columbia and 56% of segments in the Sacramento River watersheds. The simulated concentrations of doxycycline and clindamycin exceeded Le Page framework limits in 11% and 3% of segments in Columbia River, respectively, and in 15% of segments in Sacramento River. Simulated trimethoprim concentrations exceeded the Larsson framework limit in 4% of segments in Columbia and 15% of segments in Sacramento River waters. Moxifloxacin was the only antibiotic for which concentrations were estimated not to exceed framework limits in the Sacramento River and to only exceed the Larsson and Le Page framework limits in < 1% of segments in the Columbia River (Fig. 1D and Fig. 2D; Tables A1 and A2).

### 3.2.2. Comparison of mean, 90th, 95th and 99th percentile PECs to PNECs

The PEC/PNEC ratios (Tables A1 and A2) were calculated for mean, 90th, 95th and 99th percentile PECs in the Columbia (Fig. 3, S1, S3 and S4) and Sacramento (Fig. 4, S2, S5 and S6) River watersheds, modeled in low (Figs. 3 and 4, S1 and S2) and mean (Fig. S3-S6) river flow conditions and under four different antibiotic loss conditions: “All” or “None” (Fig. 3, Fig. 4, S3 and S5) and “WWTP” or “In-stream” (Fig. S1, S2, S4 and S6). The ratios < 1 indicate that simulated PECs are lower than the antibiotic levels expected to pose risks for AMR according to the frameworks and ratios > 1, in turn, indicate that PECs in watersheds are higher than the lowest AMR-promoting antibiotic concentrations. For PECs simulated under mean flow conditions, PEC/PNEC ratios  $\geq 1$  occurred only for a few antibiotics: amoxicillin, cephalixin, metronidazole and azithromycin (Table S10 and Fig. S3-S6).

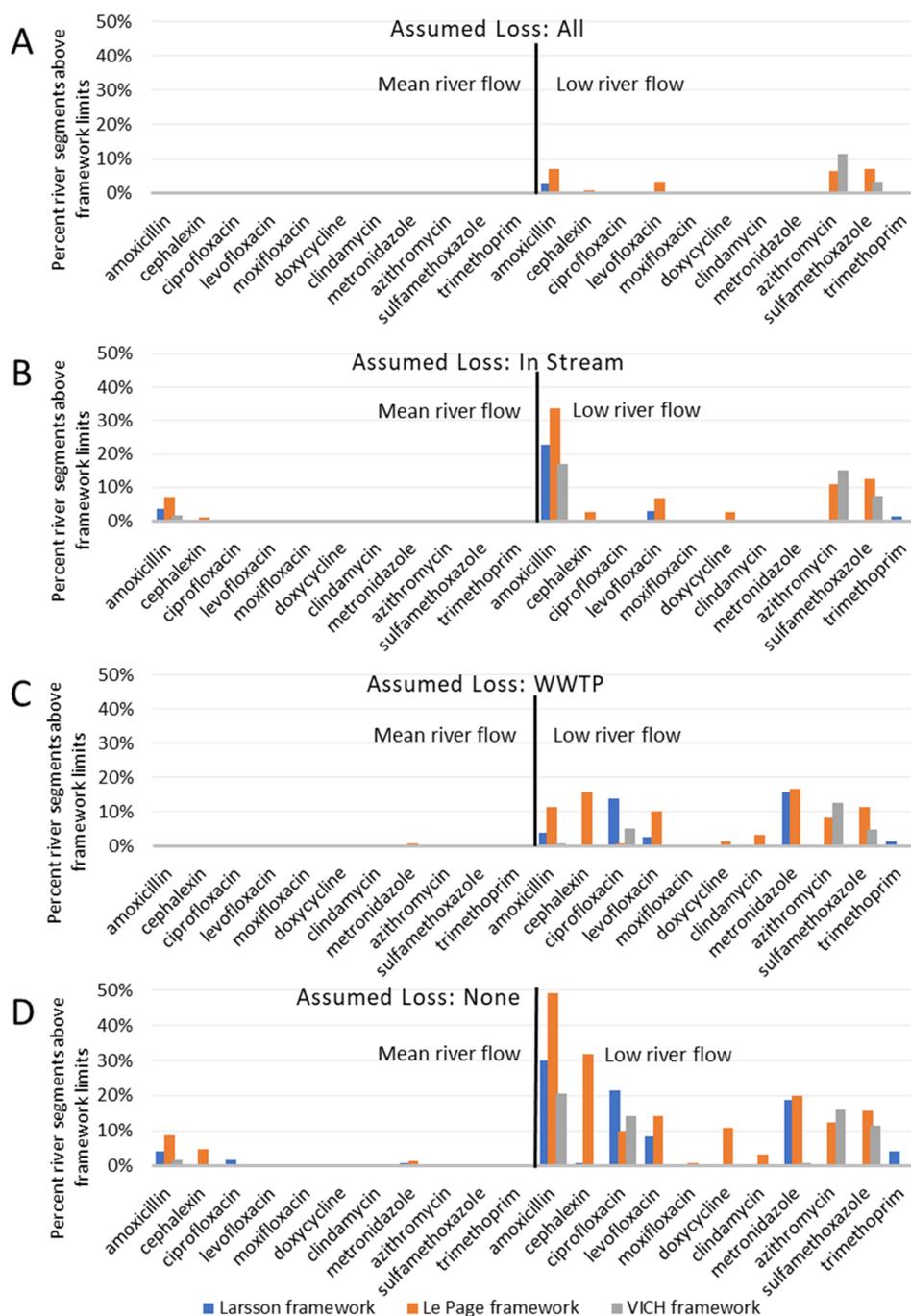
However, at more conservative conditions of low river flow, the PNECs exceeded the PECs for amoxicillin, metronidazole, ciprofloxacin, levofloxacin and trimethoprim per the Larsson framework, amoxicillin, metronidazole, cephalixin, levofloxacin, doxycycline, clindamycin, azithromycin and sulfamethoxazole per the Le Page framework, and amoxicillin, ciprofloxacin, azithromycin and sulfamethoxazole per the VICH framework (Table S10 and Fig. 3 and 4, S1 and S2).

Overall, the simulation results for the Columbia and Sacramento River watersheds indicated the same set of antibiotics having PEC values above framework limits (Table S10). Antibiotic concentrations in both watersheds at the same conditions (mean or low flow, and assumed “loss” conditions) were similar, and thus resulted in similar PEC/PNEC ratios. Overall, under mean flow conditions, the PEC/PNEC ratios were not higher than five for three out of four antibiotics which had ratios at or above one. The highest PEC/PNEC ratios were predicted for amoxicillin, both in low flow and mean flow conditions. When comparing the frameworks, applying the Le Page framework resulted in the highest PEC/PNEC ratios, while the VICH framework indicated the lowest PEC/PNEC ratios (Table S10). Also, the number of antibiotics with a PEC/PNEC ratio at or above one was highest when using the Le Page framework (eight out of 11 antibiotics in low flow conditions), and lowest when using the VICH framework PNECs (four out of 11 antibiotics in low flow conditions, Table S10).

### 3.3. Comparison of PECs of antibiotics in the Sacramento and Columbia River watersheds and PNECs of the three frameworks to MECs

To corroborate PEC-based evaluations of the frameworks, antibiotic MECs for surface waters, WWTP effluents, and groundwaters were retrieved from peer reviewed literature for comparison (Table S11). The MECs from locations throughout the world and different compartments were averaged to yield representative MECs. The MECs which were orders of magnitude higher than the rest of the reported values for the same antibiotic were excluded from the analysis. Still, reported concentrations of antibiotics varied by up to two orders of magnitude for the same antibiotic. The largest maximum concentrations were reported for sulfamethoxazole (2100  $\text{ng/L}$ ) and trimethoprim (1288  $\text{ng/L}$ ) (Anderson et al., 2012). The lowest number of MECs was available for levofloxacin and moxifloxacin (one value for each, in one publication), indicating either that the levels of these antibiotics are generally below the detection limit or analytical detection of these compounds is problematic.

The comparisons of MECs to PNECs from the Larsson, Le Page and VICH frameworks indicated that at least one MEC/PNEC ratio was > 1 for six antibiotics out of 11 according to the Larsson framework, all antibiotics except for trimethoprim according to Le Page framework and for four antibiotics out of 11 according to the VICH framework (Fig. 5). When comparing the maximum MECs and most conservative assumption PECs, both MEC/PNEC and PEC/PNEC ratios indicated either identical or a very similar set of antibiotics that exceeded the PNEC limits for each framework. Specifically, the antibiotics with environmental concentrations above Larsson threshold limits (based on both MECs and PECs) were amoxicillin, ciprofloxacin, metronidazole, and trimethoprim (Fig. 3B, Fig. 4B and Fig. 5). The MEC, but not the PEC, of azithromycin was  $\geq$  PNEC of the Larsson framework. In addition, the PEC of cephalixin (99th percentile, simulated in low flow and “no loss” conditions) was  $\geq$  the PNEC for the Columbia River only. Interestingly, the moxifloxacin MEC was above the Larsson limit while its PEC was not, and levofloxacin’s PEC was above the limit while its MEC was not. This inconsistency may derive from uncertainty in the scarce MEC data available for both antibiotics (only one value for each, Table S11). For the Le Page framework, as mentioned above, all antibiotics except for trimethoprim were above threshold limits based on MEC/PNEC values. PEC/PNEC yielded the same results as MEC/PNEC ratios with the exception of moxifloxacin which had a PEC/PNEC ratio below the Le Page limit (Fig. 3B, Fig. 4B and Fig. 5). Again, this discrepancy may have



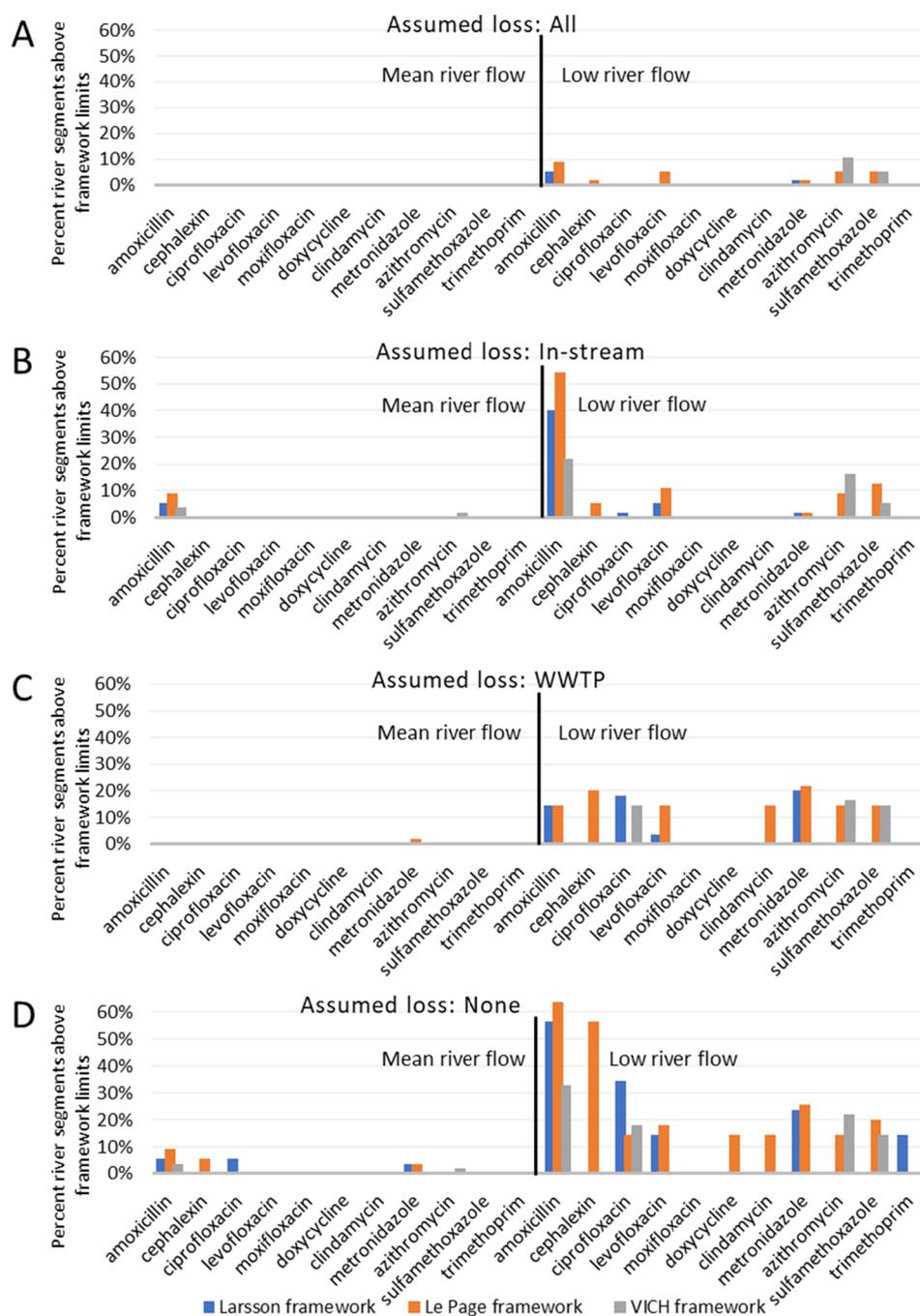
**Fig. 1.** Percent Columbia river segments (out of 750 total segments in PhATE) that had antibiotic concentrations above framework limit concentrations for each antibiotic, accounting for antibiotic losses due to (A) human metabolism, in stream degradation and wastewater treatment plant (WWTP) removal; (B) human metabolism and WWTP removal; (C) human metabolism and in-stream degradation and (D) only human metabolism. Model was run assuming either “mean river flow” (left) or “low river flow” (right). Low flow means drought conditions, i.e., 7 consecutive day low flow that occur once every 10 years.

been caused by the availability of only one MEC for moxifloxacin. For the VICH framework, both MEC/PNEC and PEC/PNEC ratios indicated the same set of antibiotics—amoxicillin, ciprofloxacin, azithromycin and sulfamethoxazole—as being above the threshold limit (Fig. 3B, Fig. 4B and Fig. 5).

**4. Discussion**

The three frameworks evaluated here each proposed PNECs, derived from either MICs or NOECs of clinically relevant or environmental bacteria, to be used as safe limit antibiotic concentrations for AMR. The estimation of PNECs relied on the assumption that AMR development

and proliferation correlates with antibiotic exposure concentrations. This assumption is based on published data showing that, in laboratory experiments, several clinically used antibiotics at extremely low concentrations, similar to the concentrations found in natural environments, could select for resistant bacteria (Gullberg et al., 2011). However, laboratory MIC tests measure acute effects on bacteria rather than chronic effects, and also measure growth inhibition under high nutrient availability. In the environment, the selection for AMR occurs during much longer timescales and the longer generation times may potentially narrow the sub-MIC selective window for many antibiotics (Bengtsson-Palme and Larsson, 2016). Further complicating the issue of environmental AMR selection is the limited knowledge of how

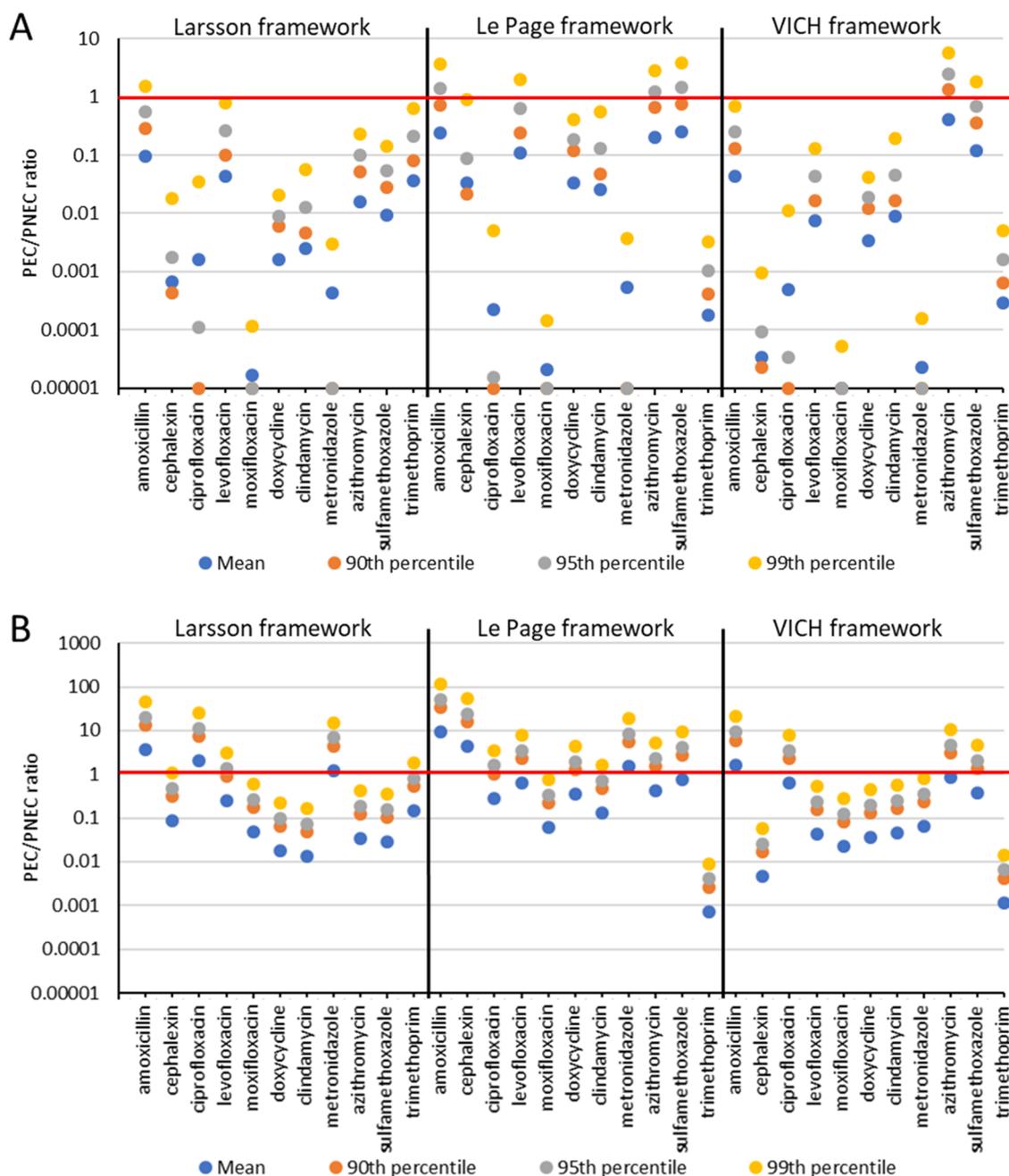


**Fig. 2.** Percent Sacramento river segments (out of 55 total segments in PhATE) that had antibiotic concentrations above framework limit concentrations for each antibiotic, accounting for antibiotic losses due to (A) human metabolism, in stream degradation and wastewater treatment plant (WWTP) removal; (B) human metabolism and WWTP removal; (C) human metabolism and in-stream degradation and (D) only human metabolism. Model was run assuming either “mean river flow” (left) or “low river flow” (right). Low flow means drought conditions, i.e., 7 consecutive day low flow that occur once every 10 years.

co-exposure to multiple antibiotics or other contaminants such as metals affects AMR development. Adding to the uncertainty are the conflicting reports of the influence of antibiotic residues on the fate of AMR. Several studies have established a positive correlation between the occurrence of antibiotics and resistant bacteria (Li et al., 2015; Rodriguez-Mozaz et al., 2015; Bengtsson-Palme et al., 2016). However, a recent European antibiotic resistance surveillance which analyzed 229 resistance genes and 25 mobile genetic elements in the influent and treated effluent of 12 WWTPs located in seven countries found no correlation between the relative abundance of AMR genes and > 50 antibiotics in the effluent samples (Parnanen et al., 2019). Also, no statistically significant correlations could be established between the

relative abundance of AMR genes in the treated effluents and country-level information on antibiotic consumption in the primary care sector (Parnanen et al., 2019).

Thus, the causes for AMR are complex and may include multiple mechanisms, such as horizontal gene transfer, genetic mutation and recombination, and selective pressures by antibiotics and other contaminants (Vikesland et al., 2017). Still, considering the urgency of managing the problem of AMR spread, mitigation practices need to be implemented proactively while the research on the mechanisms of AMR development and proliferation is ongoing. This study focused on the role of selective pressures by antibiotics on AMR and the applicability of safe limit concentrations for reducing the increased dissemination of



**Fig. 3.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Columbia River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming low river flow and accounting for antibiotic losses due to (A) human metabolism, in stream degradation and wastewater treatment plant removal, i.e. assumed loss: “All”, or (B) only human metabolism, i.e. assumed loss: “None”. Mean, 90th, 95th and 99th percentile PEC of 750 segments of the Columbia River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

AMR. The rationale behind this approach is that reduced antibiotic releases are expected to decrease selection pressure, which would result in diminished AMR bacterial populations. This study evaluated how the antibiotic threshold concentrations that are set as targets by stakeholders compare to the predicted and measured antibiotic concentrations in the surface waters to improve the understanding of the required treatment effectiveness and associated costs for reaching the targets. The study included two frameworks reported in the literature and adopted by the AMR industry alliance members (Tell et al., 2019) – the Larsson framework and the Le Page framework. Since the Le Page framework has been criticized for its overly conservative approach caused by using NOECs of environmental bacteria (cyanobacteria)

which may incur higher manufacturing costs (Bengtsson-Palme and Larsson, 2018) and the Larsson framework has received criticism for the use of MICs which have been determined in test conditions that are largely environmentally irrelevant (Le Page et al., 2018) a third approach for estimating safe antibiotic threshold limits was included for comparison in the evaluation here. Specifically, HDWCs were calculated according to VICH guidelines, which are aimed at protecting human health via managing the exposure to veterinary drugs by ingestion of drinking water and fish. Since human antibiotics have similar pathways from the production facilities to surface waters as veterinary antibiotics, the human exposure assessment methodology outlined in the VICH guidelines was deemed suitable for the purposes of this study.

An important observation that emerges from the comparison of the PNECs of the three frameworks (Table 3) is the large variation between the values proposed to be protective of AMR for the same antibiotic by different frameworks. For example, the values for cephalexin range from 80 ng/L in the Le Page framework to 76 350 ng/L in the VICH framework, and for trimethoprim from 500 ng/L in the Larsson framework to 100 000 ng/L in the Le Page framework. The differences in PNECs for the same antibiotic are in some cases up to three orders of magnitude, comparable to the range of variability between PNECs for different antibiotics. The threshold values of only two antibiotics, amoxicillin and moxifloxacin, are in the same order of magnitude in all three frameworks. The large differences exist despite the fact that all three frameworks are based on the assumption that AMR selection occurs at sub-MIC levels as per Gullberg et al. (2011). Both the Larsson and VICH frameworks use MICs of clinically relevant bacteria as a point of departure for calculations of PNECs and HDWCs, respectively. However, the VICH framework, proposed herein, differs from the Larsson framework in that it connects environmental antibiotic concentrations with human health by calculating safe antibiotic levels in drinking water and fish consumed by humans, so that PNECs (or HDWCs) would be protective of intestinal microorganisms that are sensitive to the particular antibiotic. The Le Page framework is expected to be more conservative because of using the lowest NOEC values for the most sensitive environmentally relevant phyla (cyanobacteria) as a point of departure for estimating PNECs. Indeed, in the case of most antibiotics included in this study, Le Page PNECs are lower than PNECs of the Larsson or VICH framework (Table 3). The exceptions are ciprofloxacin and trimethoprim, which have the highest PNEC values in the Le Page framework. Overall, the discrepancies in the PNECs of the three frameworks suggest that, for informed decisions on choosing the threshold limits for antibiotic releases, all available frameworks should be compared and considered.

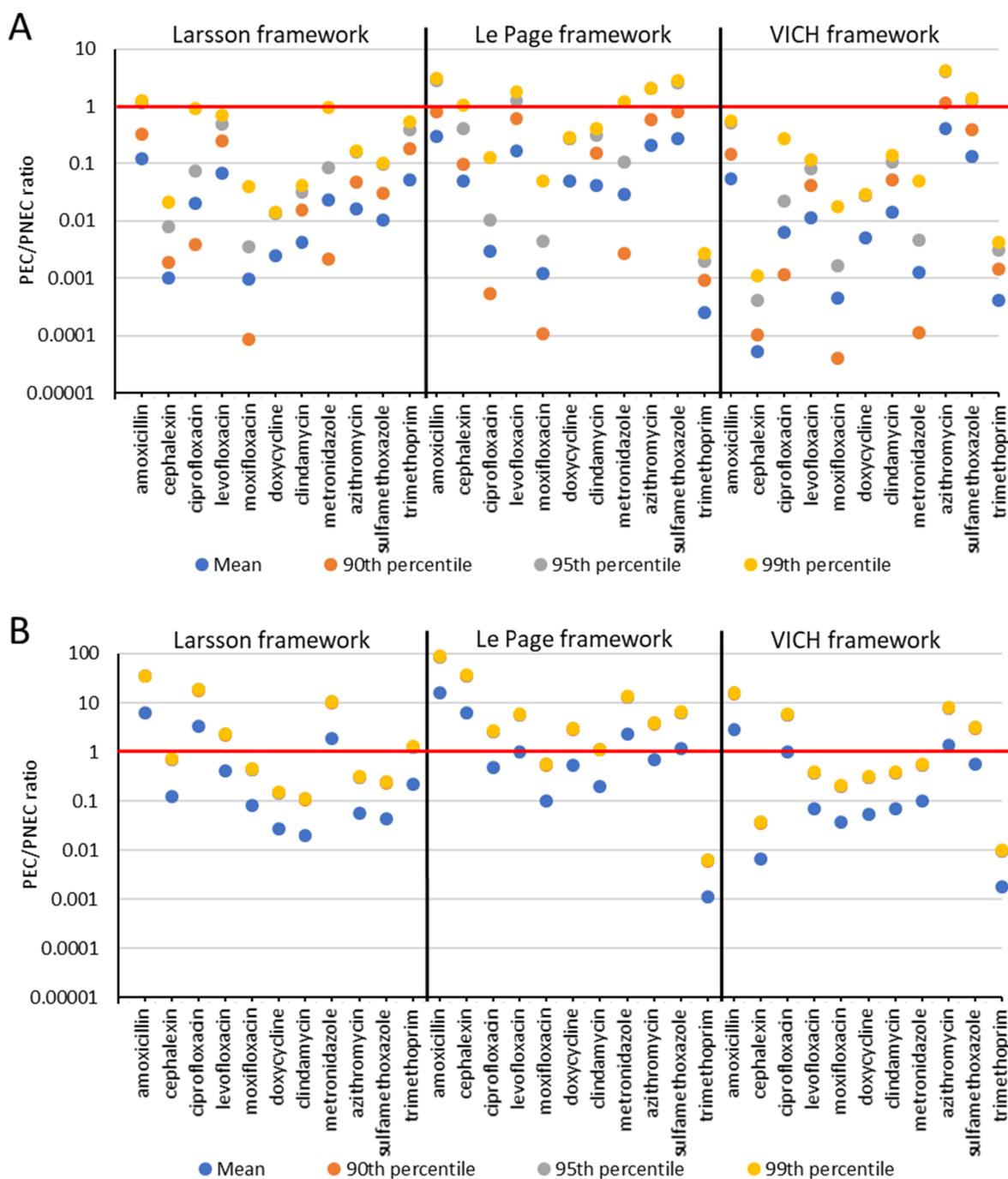
To enable comparison and evaluation of the three frameworks, PECs for 11 most sold antibiotics in the U.S. were simulated for the Sacramento and Columbia River watersheds using PhATE. The model was executed for several different conditions (assuming antibiotic loss across the WWTP, in stream, both, or no loss, each at low or mean river flow) which yielded different PEC values (Table A1 and A2). Simulations under “no loss” conditions resulted in 2–10 times higher PECs than under “all loss” conditions for most of the antibiotics. However, the simulated mean PECs of ciprofloxacin, metronidazole and moxifloxacin were three orders of magnitude higher at “no loss” settings than when assuming “all loss”. To corroborate the simulated PECs and place them in the context of antibiotic levels in the surface waters measured in different locations in the world, MECs were collected from the peer reviewed literature. When MECs and PECs were compared to PNECs of each of the three frameworks, both measured and simulated antibiotic concentrations indicated the same or a very similar set of antibiotics that exceeded the PNECs (Fig. 3B, Fig. 4B and Fig. 5). This confirmed that the PECs, simulated here for two North American rivers, were in a similar range compared to MECs reported for the same antibiotics in different geographical locations and surface water compartments (Table S11). It is worth noting that the PhATE model, used for deriving PECs, only accounted for antibiotics that resulted from human consumption, which could result in lower values than MECs that reflect all sources of antibiotics, including veterinary use. Still, PECs and MECs for several antibiotics in this study were in the same range (order of magnitude). However, since the conservative PECs for some antibiotics (moxifloxacin, doxycycline, clindamycin, azithromycin and trimethoprim) were lower than maximum reported MECs, maximum PEC values (99th percentile) obtained in conservative simulation conditions (low flow and no loss) were used when drawing conclusion on the performance of the three frameworks.

A comparison of the PEC/PNEC ratios established for the three frameworks (Table S10, Figs. 3 and 4) indicates that, expectedly, the Le Page framework is the most conservative in estimating the risk for

AMR, with the highest number of antibiotics having PEC/PNEC as well as MEC/PNEC  $\geq 1$ . The Larsson and VICH frameworks both had similar results, with five and four antibiotics, respectively, having MEC/PNEC or PEC/PNEC  $\geq 1$ . In Bengtsson-Palme and Larsson (2016), the original publication of the Larsson framework, it was established that for 28% of 111 studied antibiotics the highest reported concentrations in effluents from conventional WWTPs, as reported by Michael et al. (2013), exceeded the proposed PNECs. Additionally, the measured concentrations of ciprofloxacin in surface waters were shown to exceed not only the proposed PNECs but also the upper boundary of minimum selectable concentrations (MSCs) and reported MICs (Bengtsson-Palme and Larsson, 2016). Recently, Booth et al. (2020) compared the environmental concentrations of 12 antibiotics in municipal WWTP effluent, industrial wastewater effluent, hospital wastewater effluent, surface water, and drinking water, across 47 countries, to the PNECs for AMR selection proposed by Bengtsson-Palme and Larsson (2016). The study found that 7.9% of all reported concentrations of antibiotic residues exceeded the PNEC values while ciprofloxacin (along with clarithromycin) emerged as an antibiotic with the greatest proportion (> 30%) of residues exceeding the PNEC (Booth et al., 2020).

However, in addition to determining if antibiotic environmental concentrations are greater than proposed PNECs, analyzing the magnitude of PEC/PNEC or MEC/PNEC ratios provides additional insight into the potential impacts of the contaminating antibiotics. Specifically, AMR risk evaluation is unique in that environmental antibiotic concentrations slightly higher or equal to PNECs for AMR selection may pose a greater risk from an AMR propagation perspective than antibiotic concentrations which are orders of magnitudes higher than PNECs and closer to MIC values of the antibiotic or even bactericidal. Clearly, the proposed PNECs as well as the MICs used as departure points for PNEC calculations include uncertainties and do not accurately predict antibiotic effects to all bacterial strains and natural communities, so even at very high antibiotic concentrations AMR selection may occur in certain resistant or stress-tolerant bacteria. Still, in the case of AMR selection risk, higher PEC/PNEC or MEC/PNEC ratios do not necessarily predict greater risk for AMR propagation than lower ratios which are  $\geq 1$ . Considering that the assessment factors applied in estimating the framework PNECs were in the range of 8–10, the PEC/PNEC or MEC/PNEC ratios > 10 could be considered potentially in the range of MICs for some bacteria. Based on our results, while modeling in low flow and “All” loss scenarios did not indicate that the antibiotic PECs exceeded the PNEC > 10 times (Fig. 3A and Fig. 4A), PECs modeled in low flow and “No loss” conditions resulted in PEC/PNEC > 10 for amoxicillin (all three frameworks), metronidazole (Larsson and Le Page frameworks), ciprofloxacin (Larsson framework), cephalexin (Le Page framework) and azithromycin (VICH framework) (Fig. 3B and Fig. 4B, Table S10). MEC/PNEC > 10 occurred in the case of ciprofloxacin (Larsson framework) and azithromycin (Le Page and VICH framework) (Fig. 5). The high levels of these antibiotics in the environment are of concern not only for AMR selection risk but also for environmental risk.

Based on PEC/PNEC and MEC/PNEC  $\geq 1$ , PECs and at least one MEC of two antibiotics, amoxicillin and ciprofloxacin, were higher than their PNECs of all three frameworks (Table S10, Figs. 3–5). This indicates that the levels of these two antibiotics may be above the AMR promoting concentrations in surface waters, and that lowering their discharge levels should be a priority. Amoxicillin is a beta lactam antibiotic, a class of antibiotics known to be readily degradable by hydrolysis, both in biotic and abiotic processes (Arsand et al., 2018). Thus, due to the known lability of beta lactam structures, PhATE may overestimate amoxicillin concentrations in surface waters under “No loss” modeling scenario (Fig. 3B and Fig. 4B). Still, under assumed “WWTP”, “In-Stream” and “All” loss scenarios and also in mean flow conditions, the PEC/PNEC ratios of amoxicillin were  $\geq 1$  and in some cases the highest among the antibiotics studied across all three frameworks (Table S10). Also, even when environmental concentrations of



**Fig. 4.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Sacramento River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming low river flow and accounting for antibiotic losses due to (A) human metabolism, in stream degradation and wastewater treatment plant removal, i.e. assumed loss: "All", or (B) only human metabolism, i.e. assumed loss: "None". Mean, 90th, 95th and 99th percentile PEC of 55 segments of the Sacramento River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one. In panel B, yellow data points overlap with orange and grey data points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

amoxicillin are mostly expected to be low, hotspots can appear (Hughes et al., 2013; Table S11, Fig. 5), warranting scrutiny even for antibiotics known to be labile in the environment. This is particularly relevant when considering the reportedly high sensitivity of cyanobacteria to beta lactam antibiotics, including amoxicillin (Dias et al., 2015). Ciprofloxacin, in contrast, belongs to a class of fluoroquinolones which are resistant to biodegradation and are found in high concentrations in WWTP effluents (Mirzaei et al., 2018). In general, the highest MEC/PNEC ratio of ciprofloxacin was the highest among these of 11 antibiotics in the Larsson framework and second highest in the VICH

framework (Fig. 5). Thus, limits for fluoroquinolones should likely receive special scrutiny due to their low degradation and thus high persistence and accumulation potential in the environment. Further, a correlation between fluoroquinolone usage and resistance rates has been demonstrated (Redgrave et al., 2014), suggesting that limiting the release rates could serve as an effective measure for reducing AMR spread.

Azithromycin stands out as an antibiotic with the lowest PNECs both in the Le Page and VICH frameworks (20 and 10 ng/L, respectively; Table 3), which resulted in  $PEC/PNEC > 1$  at low flow

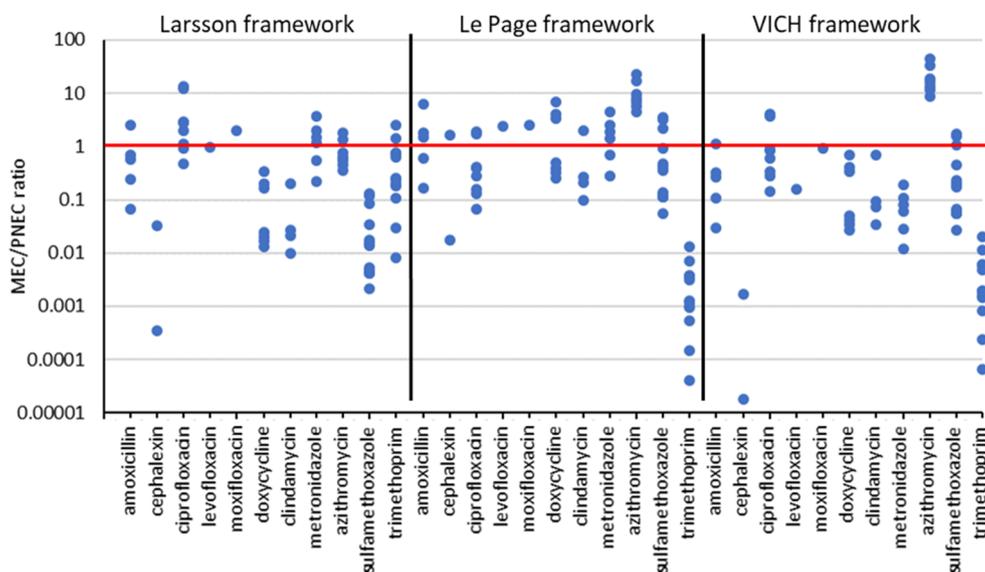


Fig. 5. Ratios of antibiotic measured environmental concentrations (MEC) and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. MEC values were collected from literature (Table S11). Red line marks MEC/PNEC ratio equal to one. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conditions (Figs. 3 and 4, Table S10) and the highest MEC/PNEC ratios for both frameworks, while all reported MECs exceeded PNECs (Fig. 5). Azithromycin is a macrolid class antibiotic and is the most hydrophobic antibiotic included in this study ( $\log K_{ow}$  4, Table S1). Due to its hydrophobicity, it adsorbs readily to sludge in WWTPs. However, negative removal rates of this antibiotic have been reported (i.e., concentrations of azithromycin have been higher in WWTP effluent than in influent; Mirzaei et al., 2018). This has been explained by certain antibiotics reverting back into their parent compound or into their original form during the treatment processes (Mirzaei et al., 2018). Additionally, azithromycin-containing effluent release from pharmaceutical manufacturing has been associated with the enrichment of macrolide-resistance genes and integrons in receiving river sediments (Milaković et al., 2019).

The Le Page and VICH frameworks both indicated sulfamethoxazole  $PEC/PNEC \geq 1$  at low flow simulation conditions. This is significant, because sulfamethoxazole is a sulfonamide which was recently reported to be among the four most frequently detected pharmaceuticals in groundwater used as a source of drinking water in the U.S. (Bexfield et al., 2019). Thus, its environmental release concentrations should likely receive special attention.

Metronidazole was among the few antibiotics (also amoxicillin, cephalexin, ciprofloxacin and azithromycin) which had PECs that were modeled in mean river flow conditions that exceeded PNECs (Table S10, Fig. S3B and S5B), and most reported metronidazole MECs exceeded the PNECs (Fig. 5). Metronidazole is a nitroimidazole, active against protozoa and mainly anaerobic bacteria, meaning that it targets both prokaryotic and eukaryotic microorganisms. Metronidazole resistance has been reported both in pathogenic bacteria (Dingsdag and Hunter, 2018) and protozoa (Rajamanikam et al., 2019) for which it is used as a first line therapy. Due to the potential for resistance development in pro- and eukaryotic microorganisms, high environmental metronidazole concentrations could pose an elevated risk for AMR proliferation.

Overall, this study indicated that simulated and measured environmental concentrations of several antibiotics in two major Western U.S. rivers may exceed concentrations predicted to have no effects on microorganisms from an AMR proliferation perspective. Among the different environmental surveillance objectives of antibiotic resistance (Huijbers et al., 2019), data on antibiotic exposure concentrations can inform risk assessment of AMR expansion, and thus comparing environmental antibiotic concentrations to PNECs for resistance selection is a feasible immediate measure, either alone or in combination with other approaches, towards managing AMR risks.

## 5. Conclusions

Here we simulated PECs of 11 most used antibiotics, which belong to nine different sub-classes, for two North-American river watersheds and compared the resulting PECs to PNECs of three frameworks which address the issue of AMR propagation. The analysis showed that PECs of all the studied antibiotics, except moxifloxacin, a 4th generation fluoroquinolone, and at least one literature reported MEC of all the studied antibiotics, were above the safe limit proposed by at least one of the three frameworks. The results indicate that a variety of different antibiotics with different mechanisms of action and physico-chemical properties may be present in environmental compartments at or above the concentrations currently proposed as safe from an AMR spread perspective. The analysis herein identified amoxicillin and ciprofloxacin as two antibiotics which exceed safe environmental limits according to all three frameworks evaluated. Concentrations of other antibiotics were also shown to be higher than PNECs at certain conditions and at varying levels. While this study was oriented towards domestic use with release into two western U.S. rivers, the approach could also guide prioritization and management control of release levels of antibiotics from manufacturing plants. While selective pressure by antibiotics is only one factor that may contribute to AMR development and proliferation, controlling the effluent antibiotic concentration is an immediately applicable measure that can be taken to act on the globally concerning issue of AMR.

## CRedit authorship contribution statement

**Monika Mortimer:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Alyssa Winchell:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Patricia A. Holden:** Conceptualization, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

## 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.

## Acknowledgement

This study was financially supported by Eli Lilly and Company. The PhATE Program software was used by permission of the Pharmaceutical Research and Manufacturers of America (PhRMA). Tiffany Tran is acknowledged for assistance with the literature search and analysis.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106053>.

## References

- Amirkia, V.D., Qiubao, P., 2011. The Antimicrobial Index: a comprehensive literature-based antimicrobial database and reference work. *Bioinformatics* 5, 365–366. <https://doi.org/10.6026/97320630005365>.
- Anderson, P., Denslow, N., Drewes, J., Livieri, A., Schlenk, D., Scott, G., Snyder, S., 2012. Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems. Southern California Coastal Water Research Project, Technical Report [https://www.waterboards.ca.gov/water\\_issues/programs/swamp/cec\\_aquatic/docs/cec\\_ecosystems\\_rpt.pdf](https://www.waterboards.ca.gov/water_issues/programs/swamp/cec_aquatic/docs/cec_ecosystems_rpt.pdf).
- Anderson, P.D., D'Aco, V.J., Shanahan, P., Chapra, S.C., Buzby, M.E., Cunningham, V.L., Duplessie, B.M., Hayes, E.P., Mastrocco, F.J., Parke, N.J., Rader, J.C., Samuelian, J.H., Schwab, B.W., 2004. Screening analysis of human pharmaceutical compounds in US surface waters. *Environ. Sci. Technol.* 38, 838–849. <https://doi.org/10.1021/es034430b>.
- Andersson, D.I., Hughes, D., 2012. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resist. Updates* 15, 162–172. <https://doi.org/10.1016/j.drug.2012.03.005>.
- Arsand, J.B., Hoff, R.B., Jank, L., Meirelles, L.N., Diaz-Cruz, M.S., Pizzolato, T.M., Barcelo, D., 2018. Transformation products of amoxicillin and ampicillin after photolysis in aqueous matrices: Identification and kinetics. *Sci. Total Environ.* 642, 954–967. <https://doi.org/10.1016/j.scitotenv.2018.06.122>.
- Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M.H., Zheng, C., 2019. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environ. Res.* 169, 483–493. <https://doi.org/10.1016/j.envres.2018.11.040>.
- Bengtsson-Palme, J., Hammaren, R., Pal, C., Ostman, M., Bjorlenius, B., Flach, C.F., Fick, J., Kristiansson, E., Tysklind, M., Larsson, D.G.J., 2016. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. *Sci. Total Environ.* 572, 697–712. <https://doi.org/10.1016/j.scitotenv.2016.06.228>.
- Bengtsson-Palme, J., Larsson, D.G.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environ. Int.* 86, 140–149. <https://doi.org/10.1016/j.envint.2015.10.015>.
- Bengtsson-Palme, J., Kristiansson, E., Larsson, D.G.J., 2018. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiol. Rev.* 42, fux053. <https://doi.org/10.1093/femsre/fux053>.
- Bengtsson-Palme, J., Larsson, D.G.J., 2018. Protection goals must guide risk assessment for antibiotics. *Environ. Int.* 111, 352–353. <https://doi.org/10.1016/j.envint.2017.10.019>.
- Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Burgmann, H., Sorum, H., Norstrom, M., Pons, M.N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., Martinez, J.L., 2015. Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol.* 13, 310–317. <https://doi.org/10.1038/nrmicro3439>.
- Bexfield, L.M., Toccalino, P.L., Belitz, K., Foreman, W.T., Furlong, E.T., 2019. Hormones and pharmaceuticals in groundwater as a source of drinking water across the United States. *Environ. Sci. Technol.* 53, 2950–2960. <https://doi.org/10.1021/acs.est.8b05592>.
- Booth, A., Aga, D.S., Wester, A.L., 2020. Retrospective analysis of the global antibiotic residues that exceed the predicted no effect concentration for antimicrobial resistance in various environmental matrices. *Environ. Int.* 141, 105796. <https://doi.org/10.1016/j.envint.2020.105796>.
- Brandt, K.K., Amézquita, A., Backhaus, T., Boxall, A., Coors, A., Heberer, T., Lawrence, J.R., Lazorchak, J., Schönfeld, J., Snape, J.R., Zhu, Y.-G., Topp, E., 2015. Ecotoxicological assessment of antibiotics: A call for improved consideration of microorganisms. *Environ. Int.* 85, 189–205. <https://doi.org/10.1016/j.envint.2015.09.013>.
- CDC, 2011. Centers for Disease Control and Prevention. Antibiotic Prescription Rates at National and State Levels in 2011. <https://gis.cdc.gov/grasp/PSA/AUMapView.html>. Accessed: July, 2018.
- Davies, J., 2009. Everything depends on everything else. *Clin. Microbiol. Infect.* 15, 1–4. <https://doi.org/10.1111/j.1469-0691.2008.02682.x>.
- Deak, D., Outterson, K., Powers, J.H., Kesselheim, A.S., 2016. Progress in the fight against multidrug-resistant bacteria? A review of U.S. Food and Drug Administration-approved antibiotics, 2010–2015. *Ann. Intern. Med.* 165, 363–372. <https://doi.org/10.7326/M16-0291>.
- Dias, E., Oliveira, M., Jones-Dias, D., Vasconcelos, V., Ferreira, E., Manageiro, V., Caniça, M., 2015. Assessing the antibiotic susceptibility of freshwater Cyanobacteria spp. *Front. Microbiol.* 6, 799. <https://doi.org/10.3389/fmicb.2015.00799>.
- Dingsdag, S.A., Hunter, N., 2018. Metronidazole: an update on metabolism, structure-cytotoxicity and resistance mechanisms. *J. Antimicrob. Chemother.* 73, 265–279. <https://doi.org/10.1093/jac/dkx351>.
- Directive, 2001/83/EC. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. *Official Journal L* – 311, 28/11/2001, p. 67 – 128.
- EMA, 2018. Guideline on the environmental risk assessment of medicinal products for human use (draft). [https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf). Accessed: November 1, 2019.
- EPA, 2000. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000). EPA-822-B-00-004.
- FDA, 2012. Drug Use Review. Accessed: July, 2018. <https://www.fda.gov/media/84216/download>.
- Grenni, P., Ancona, V., Caracciolo, A.B., 2018. Ecological effects of antibiotics on natural ecosystems: A review. *Microchem. J.* 136, 25–39. <https://doi.org/10.1016/j.micro.2017.02.006>.
- Gullberg, E., Cao, S., Berg, O.G., Ilback, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. *Plos Pathogens* 7, e1002158. <https://doi.org/10.1371/journal.ppat.1002158>.
- Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. *Environ. Sci. Technol.* 47, 661–677. <https://doi.org/10.1021/es3030148>.
- Huijbers, P.M.C., Flach, C.-F., Larsson, D.G.J., 2019. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. *Environ. Int.* 130, 104880. <https://doi.org/10.1016/j.envint.2019.05.074>.
- IFPMA, 2018. AMR Industry Alliance Antibiotic Discharge Targets. [https://www.amr-industryalliance.org/wp-content/uploads/2018/09/AMR\\_Industry\\_Alliance\\_List-of-Predicted-No-Effect-Concentrations-PNECs.pdf](https://www.amr-industryalliance.org/wp-content/uploads/2018/09/AMR_Industry_Alliance_List-of-Predicted-No-Effect-Concentrations-PNECs.pdf).
- Le Page, G., Gunnarsson, L., Snape, J., Tyler, C.R., 2017. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environ. Int.* 109, 155–169. <https://doi.org/10.1016/j.envint.2017.09.013>.
- Le Page, G., Gunnarsson, L., Snape, J., Tyler, C.R., 2018. Antibiotic risk assessment needs to protect both environmental and human health. *Environ. Int.* 115, 397–399. <https://doi.org/10.1016/j.envint.2018.03.032>.
- Lee, D., Choi, K., 2019. Comparison of regulatory frameworks of environmental risk assessments for human pharmaceuticals in EU, USA, and Canada. *Sci. Total Environ.* 671, 1026–1035. <https://doi.org/10.1016/j.scitotenv.2019.03.372>.
- Li, J.A., Cheng, W.X., Xu, L.K., Strong, P.J., Chen, H., 2015. Antibiotic-resistant genes and antibiotic-resistant bacteria in the effluent of urban residential areas, hospitals, and a municipal wastewater treatment plant system. *Environ. Sci. Pollut. Res.* 22, 4587–4596. <https://doi.org/10.1007/s11356-014-3665-2>.
- Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Res.* 47, 957–995. <https://doi.org/10.1016/j.watres.2012.11.027>.
- Milaković, M., Vestergaard, G., González-Plaza, J.J., Petrić, I., Šimatović, A., Senta, I., Kublik, S., Schlöter, M., Smalla, K., Udiković-Kolić, N., 2019. Pollution from azithromycin-manufacturing promotes macrolide-resistance gene propagation and induces spatial and seasonal bacterial community shifts in receiving river sediments. *Environ. Int.* 123, 501–511. <https://doi.org/10.1016/j.envint.2018.12.050>.
- Mirzaei, R., Yunesian, M., Nasser, S., Gholami, M., Jalilzadeh, E., Shoebi, S., Mesdaghinia, A., 2018. Occurrence and fate of most prescribed antibiotics in different water environments of Tehran, Iran. *Sci. Total Environ.* 619, 446–459. <https://doi.org/10.1016/j.scitotenv.2017.07.272>.
- Parnanen, K.M.M., Narciso-da-Rocha, C., Kneis, D., Berendonk, T.U., Cacace, D., Do, T.T., Elpers, C., Fatta-Kassinos, D., Henriques, I., Jaeger, T., Karkman, A., Martinez, J.L., Michael, S.G., Michael-Kordatou, I., O'Sullivan, K., Rodriguez-Mozaz, S., Schwartz, T., Sheng, H.J., Sorum, H., Stedfeld, R.D., Tiedje, J.M., Della Giustina, S.V., Walsh, F., Vaz-Moreira, I., Virta, M., Manaia, C.M., 2019. Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. *Sci. Adv.* 5, eaau9124. <https://doi.org/10.1126/sciadv.aau9124>.
- Rajamanikam, A., Hooi, H.S., Kudva, M., Samudi, C., Kumar, S., 2019. Resistance towards metronidazole in *Blastocystis* sp.: A pathogenic consequence. *PLoS One* 14, e0212542. <https://doi.org/10.1371/journal.pone.0212542>.
- Redgrave, L.S., Sutton, S.B., Webber, M.A., Piddock, L.J.V., 2014. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol.* 22, 438–445. <https://doi.org/10.1016/j.tim.2014.04.007>.
- Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sanchez-Melsio, A., Borrego, C.M., Barcelo, D., Balcazar, J.L., 2015. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Res.* 69, 234–242. <https://doi.org/10.1016/j.watres.2014.11.021>.
- Schwab, B.W., Hayes, E.P., Fiori, J.M., Mastrocco, F.J., Roden, N.M., Cragin, D., Meyerhoff, R.D., D'Aco, V.J., Anderson, P.D., 2005. Human pharmaceuticals in US surface waters: A human health risk assessment. *Regul. Toxicol. Pharm.* 42, 296–312. <https://doi.org/10.1016/j.yrtph.2005.05.005>.
- Seiler, C., Berendonk, T.U., 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front. Microbiol.* 3, 399. <https://doi.org/10.3389/fmicb.2012.00399>.
- Tell, J., Caldwell, D.J., Häner, A., Hellstern, J., Hoeger, B., Journal, R., Mastrocco, F., Ryan, J.J., Snape, J., Straub, J.O., Vestel, J., 2019. Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations. *Integr. Environ. Assess. Manage.* 15, 312–319. <https://doi.org/10.1002/ieam.4141>.

- Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. *Sci. Total Environ.* 429, 123–155. <https://doi.org/10.1016/j.scitotenv.2012.04.028>.
- VICH, 2012. VICH GL36(R) Guidance for Industry. Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine: 2012.
- Vikesland, P.J., Pruden, A., Alvarez, P.J.J., Aga, D., Bürgmann, H., Li, X.-D., Manaia, C.M., Nambi, I., Wigginton, K., Zhang, T., Zhu, Y.-G., 2017. Toward a comprehensive strategy to mitigate dissemination of environmental sources of antibiotic resistance. *Environ. Sci. Technol.* 51, 13061–13069. <https://doi.org/10.1021/acs.est.7b03623>.
- Xiong, W., Sun, Y., Ding, X., Wang, M., Zeng, Z., 2015. Selective pressure of antibiotics on ARGs and bacterial communities in manure-polluted freshwater-sediment microcosms. *Front. Microbiol.* 6, 194. <https://doi.org/10.3389/fmicb.2015.00194>.
- Zaranyika, M.F., Dzomba, P., Kugara, J., 2015. Speciation and persistence of doxycycline in the aquatic environment: characterization in terms of steady state kinetics. *J. Environ. Sci. Health Part B - Pesticides Food Contam. Agric. Wastes* 50, 908–918. <https://doi.org/10.1080/03601234.2015.1067101>.

## Supporting Information

### Evaluation of frameworks proposed as protective of antimicrobial resistance propagation in the environment

Monika Mortimer,<sup>a,b\*</sup> Alyssa Winchell,<sup>b</sup> Patricia A. Holden<sup>b\*</sup>

<sup>a</sup>Institute of Environmental and Health Sciences, College of Quality and Safety Engineering, China Jiliang University, Hangzhou, Zhejiang 310018, China

<sup>b</sup>Bren School of Environmental Science and Management and Earth Research Institute, University of California Santa Barbara, Santa Barbara, California 93106, United States

\*M. Mortimer, Room 308-2, Fangzheng Building, China Jiliang University, 168 Xueyuan Street, Hangzhou, Zhejiang 310018, China

\*P. A. Holden, 3508 Bren Hall, Bren School of Environmental Science & Management, University of California, Santa Barbara, CA 93106-5131 USA

E-mail addresses:

M. Mortimer: [mmortimer@cjl.u.edu.cn](mailto:mmortimer@cjl.u.edu.cn)

P. A. Holden: [holden@bren.ucsb.edu](mailto:holden@bren.ucsb.edu)

**Table S1.** Physico-chemical properties of antibiotics.

| <b>Antibiotic</b> | <b>Chemical type at pH7</b> | <b>Water solubility (mg/L)</b>  | <b>pKa<sub>1</sub></b> | <b>Biodegradation rate coefficient (1/h)<sup>v</sup></b> | <b>logK<sub>ow</sub></b> |
|-------------------|-----------------------------|---------------------------------|------------------------|--|--------------------------|
| amoxicillin       | zwitterion <sup>a</sup>     | 4000 <sup>l</sup>               | 2.8 <sup>a</sup>       | 0.9  | -1.56 <sup>w</sup>       |
| cephalexin        | zwitterion <sup>b</sup>     | 13500 <sup>l</sup>              | 2.6 <sup>q</sup>       | 1.8  | -2.14 <sup>x</sup>       |
| ciprofloxacin     | zwitterion <sup>c</sup>     | 10000 <sup>l</sup>              | 2.9 <sup>r</sup>       | 0.3  | 0.28 <sup>y</sup>        |
| levofloxacin      | zwitterion <sup>d</sup>     | insoluble in water <sup>m</sup> | 5.7 <sup>d</sup>       | 0.1  | 0.51 <sup>x</sup>        |
| moxifloxacin      | zwitterion <sup>e</sup>     | 19600 <sup>n</sup>              | 8.9 <sup>s</sup>       | 0.1  | -0.28 <sup>z</sup>       |
| doxycycline       | neutral <sup>f</sup>        | 100 <sup>l</sup>                | 3.7 <sup>r</sup>       | 0.07   | -3.19 <sup>x</sup>       |
| clindamycin       | base <sup>g</sup>           | 100000 <sup>l</sup>             | 7.7 <sup>t</sup>       | 0.5  | 1.04 <sup>x</sup>        |
| azithromycin      | base <sup>h</sup>           | 2.37 <sup>o</sup>               | 8.96 <sup>u</sup>      | 0.01   | 4.02 <sup>A</sup>        |
| metronidazole     | base <sup>i</sup>           | 10000 <sup>l</sup>              | 2.4 <sup>q</sup>       | 0.1  | -0.22 <sup>B</sup>       |
| sulfamethoxazole  | neutral <sup>j</sup>        | 10 <sup>l</sup>                 | 1.7 <sup>r</sup>       | 0.1  | 0.86 <sup>C</sup>        |
| trimethoprim      | neutral <sup>k</sup>        | 405.3 <sup>p</sup>              | 3.2 <sup>r</sup>       | 0.1  | 0.802 <sup>D</sup>       |

<sup>a</sup>Felix et al. (2016); <sup>b</sup>Matsumoto et al. (1994); <sup>c</sup>Jin et al. (2018); <sup>d</sup>Hirano et al. (2006); <sup>e</sup>Quiming et al. (2007); <sup>f</sup>Legendre et al. (2012); <sup>g</sup>Klempner and Styrk (1983); <sup>h</sup>Aucamp et al. (2015); <sup>i</sup>Erah et al. (1997); <sup>j</sup>Kimura et al. (2004); <sup>k</sup>Im et al. (2016); <sup>l</sup>Jjemba (2006); <sup>m</sup>Gonzalez et al. (2000); <sup>n</sup>Varanda et al. (2006); <sup>o</sup>HSDB (2018); <sup>p</sup>Yalkowsky and He (2003); <sup>q</sup>Shalaeva et al. (2008); <sup>r</sup>Qiang and Adams (2004); <sup>s</sup>Refaat et al. (2016); <sup>t</sup>Wan et al. (2003); <sup>u</sup>Zrnčić et al. (2015); <sup>v</sup>Obach et al. (2008); <sup>w</sup>Winiwarter et al. (1998); <sup>x</sup>Viswanadhan et al. (1989); <sup>y</sup>Takács-Novák et al. (1992); <sup>z</sup>Langlois et al. (2005); <sup>A</sup>Lombardo et al. (2000); <sup>B</sup>McFarland et al. (1997); <sup>C</sup>Rafols et al. (2017); <sup>D</sup>Kansy (2007).

**Table S2.** Antibiotic prescription rates (number of prescriptions per 1000 persons) at national and state levels in 2011(CDC, 2011).

| <b>Antibiotic</b> | <b>Corresponding antibiotic class in CDC database</b> | <b>National</b> | <b>California</b> | <b>Oregon</b> | <b>Washington</b> | <b>Idaho</b> | <b>Montana</b> |
|-------------------|---|-----------------|-------------------|---------------|-------------------|--------------|----------------|
| amoxicillin       | Penicillins   | 196             | 156               | 147           | 142               | 171          | 159            |
| cephalexin        | Cephalosporins  | 117             | 77                | 73            | 87                | 108          | 84             |
| ciprofloxacin     | Fluoroquinolones                                      | 101             | 71                | 73            | 67                | 71           | 77             |
| levofloxacin      | Fluoroquinolones                                      | 101             | 71                | 73            | 67                | 71           | 77             |
| moxifloxacin      | Fluoroquinolones                                      | 101             | 71                | 73            | 67                | 71           | 77             |
| doxycycline       | All classes   | 877             | 629               | 636           | 662               | 740          | 724            |
| clindamycin       | All classes   | 877             | 629               | 636           | 662               | 740          | 724            |
| azithromycin      | Macrolides  | 190             | 135               | 120           | 136               | 142          | 163            |
| metronidazole     | All classes   | 877             | 629               | 636           | 662               | 740          | 724            |
| sulfamethoxazole  | All classes   | 877             | 629               | 636           | 662               | 740          | 724            |
| trimethoprim      | All classes   | 877             | 629               | 636           | 662               | 740          | 724            |

CDC - Centers for Disease Control and Prevention

**Table S3.** Estimated antibiotic sales in kilograms (kg) in Sacramento and Columbia watershed areas in 2011.<sup>1</sup>

| <b>Antibiotic</b> | <b>Sacramento</b> | <b>Columbia</b> |
|-------------------|-------------------|-----------------|
| amoxicillin       | 908,079           | 862,736         |
| cephalexin        | 196,255           | 217,139         |
| ciprofloxacin     | 147,506           | 145,399         |
| levofloxacin      | 39,245            | 38,684          |
| moxifloxacin      | 7,735             | 7,624           |
| doxycycline       | 46,588            | 49,506          |
| clindamycin       | 51,047            | 54,245          |
| azithromycin      | 74,249            | 73,743          |
| metronidazole     | 86,081            | 91,474          |
| sulfamethoxazole  | 285,725           | 303,625         |
| trimethoprim      | 58,313            | 61,966          |

<sup>1</sup>Data was calculated based on prescription rates at national and state levels (Table S2), national sales data (Table 1), and populations of four states in the Columbia watershed (Table S4).

**Table S4.** Populations in the Columbia River Watershed States in 2010 (U.S. Census Bureau).

| <b>State</b> | <b>Population</b> |
|--------------|-------------------|
| Oregon       | 3,831,074         |
| Washington   | 6,724,540         |
| Idaho        | 1,567,582         |
| Montana      | 989,415           |

**Table S5.** Fractional losses and first order loss coefficients of antibiotics by source.

| <b>Antibiotic</b> | <b>Fractional loss by human metabolism</b> | <b>Fractional loss in wastewater treatment plant</b> | <b>First order loss coefficients in stream (1/day)</b> |
|-------------------|--|--|--|
| amoxicillin       | 0.4  | 0.96 <sup>a</sup>                                    | 0.035 <sup>d</sup>                                     |
| cephalexin        | 0.1  | 0.82 <sup>a</sup>                                    | 2.9 <sup>e</sup>                                       |
| ciprofloxacin     | 0.5  | 0.7 <sup>a</sup>                                     | 63 <sup>f</sup>  |
| levofloxacin      | 0.09                                       | 0.55 <sup>b</sup>                                    | 0.14 <sup>g</sup>                                      |
| moxifloxacin      | 0.55                                       | 0.4 <sup>c</sup>                                     | 490 <sup>h</sup>                                       |
| doxycycline       | 0.6  | 0.71 <sup>a</sup>                                    | 67.6% <sup>i</sup>                                     |
| clindamycin       | 0.86                                       | -1.5 <sup>a</sup>                                    | 0.43 <sup>j</sup>                                      |
| azithromycin      | 0.94                                       | 0.44 <sup>a</sup>                                    | 0.0084 <sup>k</sup>                                    |
| metronidazole     | 0.05                                       | 0.38 <sup>a</sup>                                    | 145 <sup>l</sup>                                       |
| sulfamethoxazole  | 0.16                                       | 0.52 <sup>a</sup>                                    | 0.032 <sup>j</sup>                                     |
| trimethoprim      | 0.33                                       | 0.4 <sup>a</sup>                                     | 0.12 <sup>g</sup>                                      |

<sup>a</sup>Verlicchi et al. (2012); <sup>b</sup>Park et al. (2017); <sup>c</sup>Jia et al. (2012); <sup>d</sup>Braschi et al. (2013); <sup>e</sup>Yan et al. (2017); <sup>f</sup>Wang et al. (2018); <sup>g</sup>Lam et al. (2004); <sup>h</sup>Sturini et al. (2012); <sup>i</sup>Zaranyika et al. (2015); <sup>j</sup>Henzler et al. (2014); <sup>k</sup>Vermillion Maier and Tjeerdema (2018); <sup>l</sup>Tong et al. (2011)

**Table S6.** Minimum inhibitory concentrations at which growth of 50% of the bacterial isolates is inhibited (MIC<sub>50</sub>, µg/mL)<sup>1</sup> for the relevant intestinal microflora described in VICH (2012).

| <b>Genera</b>             | <b>amoxi-<br/>cillin</b> | <b>cepha-<br/>lexin</b> | <b>cipro-<br/>floxacin</b> | <b>levo-<br/>floxacin</b> | <b>moxi-<br/>floxacin</b> | <b>doxy-<br/>cycline</b> | <b>clinda-<br/>mycin</b> | <b>azithro-<br/>mycin</b> | <b>metro-<br/>nidazole</b> | <b>sulfa-<br/>methox-<br/>azole</b> | <b>trimetho-<br/>prim</b> |
|---------------------------|--------------------------|-------------------------|----------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|----------------------------|-------------------------------------|---------------------------|
| <i>Bacteroides</i>        | 2                        | 32                      | 0.5                        | 0.06                      | 0.125                     | 0.25                     | ≤ 0.015                  | 0.06                      | 0.125                      | NA                                  | NA                        |
| <i>Bifidobacterium</i>    | 0.25                     | NA                      | 1                          | 0.5                       | 0.125                     | 0.25                     | 0.015                    | 0.03                      | 2                          | NA                                  | NA                        |
| <i>Clostridium</i>        | ≤ 0.125                  | 32                      | 1                          | 0.125                     | 0.25                      | 8                        | ≤ 0.03                   | 0.25                      | 0.06                       | NA                                  | NA                        |
| <i>Enterococcus</i>       | 0.5                      | 32                      | 0.5                        | 0.25                      | 0.125                     | 0.12                     | 2                        | 1                         | > 1024                     | NA                                  | NA                        |
| <i>Escherichia coli</i>   | 6.25                     | 4                       | 0.008                      | 0.015                     | 0.03                      | 1                        | 2                        | 4                         | NA                         | > 0.06                              | 0.5                       |
| <i>Eubacterium</i>        | 1                        | NA                      | 0.5                        | 0.5                       | 0.25                      | NA                       | 0.015                    | ≤ 0.03                    | 0.06                       | NA                                  | NA                        |
| <i>Fusobacterium</i>      | ≤ 0.125                  | NA                      | 1                          | 0.5                       | 0.125                     | 0.25                     | 0.032                    | 0.125                     | ≤ 0.125                    | NA                                  | NA                        |
| <i>Lactobacillus</i>      | 1                        | NA                      | 0.5                        | 0.5                       | 0.25                      | 0.25                     | 0.03                     | 0.03                      | > 8                        | NA                                  | NA                        |
| <i>Peptococcus</i>        | NA                       | 32                      | 0.5                        | NA                        | NA                        | NA                       | 0.125                    | NA                        | 1                          | NA                                  | NA                        |
| <i>Peptostreptococcus</i> | ≤ 0.125                  | 32                      | 0.5                        | 0.06                      | 0.12                      | NA                       | 0.03                     | 0.06                      | 0.25                       | NA                                  | NA                        |

<sup>1</sup>Data are from the Antimicrobial Index Knowledgebase ([http://antibiotics.toku-e.com/antimicrobial\\_528\\_26.html](http://antibiotics.toku-e.com/antimicrobial_528_26.html), accessed: Feb. 12, 2019).

NA – no value available

**Table S7.** Minimal minimum inhibitory concentrations (MIC<sub>min</sub>, µg/mL)<sup>1</sup> for bacterial species sensitive to selected antibiotics as listed in drug datasheets.

| <b>amoxicillin</b>  |                          | <b>cephalexin</b>   |                          | <b>doxycycline</b>   |                          |
|---|--------------------------|---|--------------------------|--|--------------------------|
| <b>Sensitive strains</b>  | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>  | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>                                   | <b>MIC<sub>min</sub></b> |
| <i>Enterococcus faecalis</i>                                      | 0.25                     | <i>Staphylococcus aureus</i><br>(including penicillinase-producing strains) | 0.78                     | <i>Ureaplasma urealyticum</i><br>(doxycycline susceptible) | 0.06                     |
| <i>Staphylococcus</i> spp. (β-lactamase-negative strains only)    | 0.1                      | <i>Staphylococcus epidermidis</i><br>(penicillin-susceptible strains)       | 0.5                      | <i>Mycoplasma pneumoniae</i>                               | 0.03                     |
| <i>Streptococcus pneumoniae</i>                                   | 0.008                    | <i>Streptococcus pneumoniae</i>   | 0.5                      | <i>Chlamydia pneumoniae</i>                                | 0.016                    |
| <i>Streptococcus pyogenes</i>                                     | 0.03                     | <i>Streptococcus pyogenes</i>   | 0.2                      | <i>Haemophilus influenzae</i>                              | 0.5                      |
| <i>Escherichia coli</i> (β-lactamase-negative strains only)       | 1.56                     | <i>Escherichia coli</i>   | 2                        | <i>Klebsiella species</i>                                  | 0.5                      |
| <i>Haemophilus influenzae</i> (β-lactamase-negative strains only) | 0.125                    | <i>Haemophilus influenzae</i>   | 4                        | <i>Streptococcus pneumoniae</i>                            | 0.12                     |
| <i>Proteus mirabilis</i> (β-lactamase-negative strains only)      | 0.5                      | <i>Klebsiella pneumoniae</i>  | 0.78                     | <i>Borrelia</i>  | 0.06                     |
| <i>Helicobacter pylori</i>  | 0.0005                   | <i>Proteus mirabilis</i>  | 1.6                      | <i>Campylobacter jejuni</i> (Finland)                      | 0.06                     |
| <i>Moraxella catarrhalis</i>                                      | 0.008                    |   |                          | <i>Brucella</i>  | 0.128                    |
|   |                          |   |                          | <i>Escherichia coli</i>                                    | 0.25                     |
|   |                          |   |                          | <i>Enterobacter aerogenes</i>                              | 0.12                     |
|   |                          |   |                          | <i>Fusobacterium spp.</i>                                  | 0.032                    |
|   |                          |   |                          | <i>Clostridium</i>   | 4                        |

<sup>1</sup>Values are from the Antimicrobial Index Knowledgebase, <http://antibiotics.toku-e.com/>, accessed: Feb. 12, 2019. Only sensitive strains that had MIC<sub>min</sub> data available are included in the table.

**Table S7, continued.**

| <b>ciprofloxacin</b>                                       |                          | <b>levofloxacin</b>                     |                          | <b>moxifloxacin</b>   |                          |
|--|--------------------------|---|--------------------------|---|--------------------------|
| <b>Sensitive strains</b>                                   | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>                | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>                                      | <b>MIC<sub>min</sub></b> |
| <i>Aeromonas hydrophila</i>                                | 0.008                    | <i>Chlamydophila pneumoniae</i>         | 0.5                      | <i>Chlamydia pneumoniae</i>                                   | 0.125                    |
| <i>Campylobacter jejuni</i>                                | 0.03                     | <i>Citrobacter freundii</i>             | 0.00625                  | <i>Citrobacter freundii</i>                                   | 0.03                     |
| <i>Citrobacter diversus</i>                                | 0.008                    | <i>Citrobacter koseri</i>               | 0.015                    | <i>Enterobacter cloacae</i>                                   | 0.015                    |
| <i>Citrobacter freundii</i>                                | 0.004                    | <i>Clostridium perfringens</i>          | 0.12                     | <i>Escherichia coli</i>                                       | 0.004                    |
| <i>Enterobacter aerogenes</i>                              | 0.008                    | <i>Enterobacter aerogenes</i>           | 0.008                    | <i>Fusobacterium species</i>                                  | 0.06                     |
| <i>Enterobacter cloacae</i>                                | 0.00625                  | <i>Enterobacter cloacae</i>             | 0.008                    | <i>Haemophilus influenzae</i>                                 | 0.004                    |
| <i>Enterococcus faecalis</i>                               | 0.25                     | <i>Enterobacter sakazakii</i>           | 0.25                     | <i>Haemophilus parainfluenzae</i>                             | 0.016                    |
| <i>Escherichia coli</i>                                    | 0.002                    | <i>Enterococcus faecalis</i>            | 0.006                    | <i>Klebsiella oxytoca</i>                                     | 0.03                     |
| <i>Haemophilus influenzae</i>                              | 0.004                    | <i>Escherichia coli</i>                 | 0.004                    | <i>Klebsiella pneumoniae</i>                                  | 0.008                    |
| <i>Haemophilus parainfluenzae</i>                          | 0.008                    | <i>Haemophilus influenzae</i>           | 0.006                    | <i>Legionella pneumophila</i>                                 | 0.004                    |
| <i>Klebsiella pneumoniae</i>                               | 0.008                    | <i>Haemophilus parainfluenzae</i>       | 0.006                    | <i>Moraxella catarrhalis</i>                                  | 0.008                    |
| <i>Legionella pneumophila</i>                              | 0.008                    | <i>Klebsiella oxytoca</i>               | 0.015                    | <i>Mycoplasma pneumoniae</i>                                  | 0.016                    |
| <i>Moraxella catarrhalis</i>                               | 0.004                    | <i>Klebsiella pneumoniae</i>            | 0.008                    | <i>Peptostreptococcus species</i>                             | 0.03                     |
| <i>Morganella morganii</i>                                 | 0.00625                  | <i>Legionella pneumophila</i>           | 0.002                    | <i>Prevotella species</i>                                     | 0.03                     |
| <i>Neisseria gonorrhoeae</i>                               | 0.004                    | <i>Moraxella catarrhalis</i>            | 0.006                    | <i>Proteus mirabilis</i>                                      | 0.06                     |
| <i>Pasteurella multocida</i>                               | 0.008                    | <i>Morganella morganii</i>              | 0.0125                   | <i>Staphylococcus aureus</i>                                  | 0.016                    |
| <i>Proteus mirabilis</i>                                   | 0.008                    | <i>Mycoplasma pneumoniae</i>            | 0.063                    | <i>Staphylococcus epidermidis</i>                             | 0.008                    |
| <i>Proteus vulgaris</i>                                    | 0.008                    | <i>Pantoea agglomerans</i>              | 0.015                    | <i>Streptococcus agalactiae</i>                               | 0.03                     |
| <i>Providencia rettgeri</i>                                | 0.00625                  | <i>Proteus mirabilis</i>                | 0.015                    | <i>Streptococcus pneumoniae</i>                               | 0.03                     |
| <i>Providencia stuartii</i>                                | 0.008                    | <i>Proteus vulgaris</i>                 | 0.0125                   | <i>Streptococcus pneumoniae</i><br>(levofloxacin-susceptible) | 0.03                     |
| <i>Pseudomonas aeruginosa</i>                              | 0.03                     | <i>Providencia rettgeri</i>             | 0.025                    | <i>Streptococcus pyogenes</i>                                 | 0.06                     |
| <i>Salmonella enteritidis</i>                              | 0.00313                  | <i>Providencia stuartii</i>             | 0.06                     | <i>Streptococcus spp. (Viridans group)</i>                    | 0.12                     |
| <i>Salmonella typhi</i>                                    | 0.008                    | <i>Pseudomonas aeruginosa*</i>          | 0.008                    |   |                          |
| <i>Shigella boydii</i>                                     | 0.008                    | <i>Serratia marcescens</i>              | 0.015                    |   |                          |
| <i>Shigella dysenteriae</i>                                | 0.008                    | <i>Staphylococcus aureus</i>            | 0.006                    |   |                          |
| <i>Shigella flexneri</i>                                   | 0.008                    | <i>Staphylococcus epidermidis</i>       | 0.006                    |   |                          |
| <i>Shigella sonnei</i>                                     | 0.004                    | <i>Staphylococcus haemolyticus</i>      | 0.03                     |   |                          |
| <i>Staphylococcus aureus</i>                               | 0.06                     | <i>Staphylococcus saprophyticus</i>     | 0.03                     |   |                          |
| <i>Staphylococcus epidermidis</i>                          | 0.0006                   | <i>Streptococcus agalactiae</i>         | 0.06                     |   |                          |
| <i>Staphylococcus haemolyticus</i>                         | 0.015                    | <i>Streptococcus milleri</i>            | 0.25                     |   |                          |
| <i>Staphylococcus hominis</i>                              | 0.06                     | <i>Streptococcus pneumoniae</i>         | 0.03                     |   |                          |
| <i>Staphylococcus saprophyticus</i>                        | 0.06                     | <i>Streptococcus pyogenes</i>           | 0.025                    |   |                          |
| <i>Streptococcus pneumoniae</i> (levofloxacin-susceptible) | 0.06                     | <i>Viridans group streptococci</i>      | 0.03                     |   |                          |
| <i>Streptococcus pyogenes</i>                              | 0.0125                   | $\beta$ -hemolytic <i>Streptococcus</i> | 0.06                     |   |                          |
| <i>Vibrio cholerae</i>                                     | 10                       |   |                          |   |                          |
| <i>Vibrio parahaemolyticus</i>                             | 10                       |   |                          |   |                          |
| <i>Yersinia enterocolitica</i>                             | 0.008                    |   |                          |   |                          |
| <i>zae Serratia marcescens</i>                             | 0.008                    |   |                          |   |                          |

**Table S7, continued.**

| <b>clindamycin</b>  |                          | <b>azithromycin</b>  |                          | <b>metronidazole</b>                          |                          |
|---|--------------------------|--|--------------------------|---|--------------------------|
| <b>Sensitive strains</b>  | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>                                   | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>                      | <b>MIC<sub>min</sub></b> |
| <i>Clostridium clostridioforme</i>  | 0.015                    | Beta-hemolytic streptococci<br>(Groups C, F, G)            | 0.06                     | <i>Bacteroides</i><br><i>thetaiotaomicron</i> | 0.2                      |
| <i>Clostridium perfringens</i>  | 0.008                    | <i>Chlamydia trachomatis</i>                               | 0.016                    | <i>Bacteroides caccae</i>                     | 0.5                      |
| <i>Eubacterium lentum</i>   | 0.03                     | <i>Chlamydophila pneumoniae</i>                            | 0.015                    | <i>Bacteroides distasonis</i>                 | 0.125                    |
| <i>Finegoldia</i><br>("Peptostreptococcus")                               | 0.03                     | <i>Haemophilus influenzae</i>                              | 0.001                    | <i>Bacteroides fragilis</i>                   | 0.06                     |
| <i>Fusobacterium</i><br><i>necrophorum</i>                                | 0.015                    | <i>Legionella pneumophila</i>                              | 0.008                    | <i>Bacteroides ovatus</i>                     | 0.25                     |
| <i>Fusobacterium nucleatum</i>  | 0.015                    | <i>Moraxella catarrhalis</i>                               | 0.008                    | <i>Bacteroides uniformis</i>                  | 0.25                     |
| <i>Micromonas</i><br>("Peptostreptococcus")                               | 0.03                     | <i>Mycoplasma pneumoniae</i>                               | 0.0006                   | <i>Bacteroides vulgatus</i>                   | 0.12                     |
| <i>Peptostreptococcus</i><br><i>anaerobius</i>                            | 0.016                    | <i>Peptostreptococcus</i>                                  | 0.03                     | <i>Clostridium</i>                            | 0.015                    |
| <i>Prevotella bivia</i>   | 0.015                    | <i>Prevotella bivia</i>                                    | 0.25                     | <i>Eubacterium</i>                            | 0.12                     |
| <i>Prevotella intermedia</i>  | 0.016                    | <i>Staphylococcus aureus</i>                               | 0.12                     | <i>Fusobacterium</i>                          | 0.008                    |
| <i>Prevotella melaninogenica</i>  | 0.016                    | <i>Streptococcus agalactiae</i>                            | 0.008                    | <i>Peptococcus</i>                            | 0.25                     |
| <i>Propionibacterium acnes</i>  | 0.03                     | <i>Streptococcus pneumoniae</i>                            | 0.008                    | <i>Peptostreptococcus</i>                     | 0.12                     |
| <i>Staphylococcus aureus</i><br>(methicillin-susceptible<br>strains)      | 0.06                     | <i>Streptococcus pyogenes</i>                              | 0.001                    | <i>Prevotella bivia</i>                       | 0.12                     |
| <i>Staphylococcus epidermidis</i><br>(methicillin-susceptible<br>strains) | 0.06                     | <i>Ureaplasma urealyticum</i><br>(doxycycline-susceptible) | 0.06                     | <i>Prevotella buccae</i>                      | 0.06                     |
| <i>Streptococcus agalactiae</i>   | 0.02                     | Viridans group streptococci                                | 0.008                    | <i>Prevotella disiens</i>                     | 0.06                     |
| <i>Streptococcus anginosus</i>  | 0.064                    |  |                          |   |                          |
| <i>Streptococcus mitis</i>  | 0.064                    |  |                          |   |                          |
| <i>Streptococcus oralis</i>   | 0.064                    |  |                          |   |                          |
| <i>Streptococcus pneumoniae</i><br>(penicillin-susceptible strains)       | 0.008                    |  |                          |   |                          |
| <i>Streptococcus pyogenes</i>   | 0.016                    |  |                          |   |                          |

**Table S7, continued.**

| <b>sulfamethoxazole</b>         |                          | <b>trimethoprim</b>             |                          |
|---------------------------------|--------------------------|---------------------------------|--------------------------|
| <b>Sensitive strains</b>        | <b>MIC<sub>min</sub></b> | <b>Sensitive strains</b>        | <b>MIC<sub>min</sub></b> |
| <i>Enterobacter</i>             | 0.03                     | <i>Enterobacter</i>             | 156                      |
| <i>Escherichia coli</i>         | 0.015                    | <i>Escherichia coli</i>         | 0.062                    |
| <i>Haemophilus influenzae</i>   | 0.06                     | <i>Haemophilus influenzae</i>   | 0.25                     |
| <i>Klebsiella</i>               | 0.06                     | <i>Klebsiella</i>               | 0.25                     |
| <i>Streptococcus pneumoniae</i> | 152                      | <i>Morganella morganii</i>      | 156                      |
|                                 |                          | <i>Proteus vulgaris</i>         | 156                      |
|                                 |                          | <i>Shigella flexneri</i>        | 156                      |
|                                 |                          | <i>Shigella sonnei</i>          | 9.8                      |
|                                 |                          | <i>Streptococcus pneumoniae</i> | 2                        |

**Table S8.** Calculated minimum inhibitory concentrations ( $MIC_{calc}$ ), calculated no-observable adverse effect concentrations ( $NOAEC_{calc}$ ) and respective microbiological acceptable daily intake (ADI) values for selected antibiotics for human intestinal microflora.

| <b>Antibiotic</b> | <b><math>MIC_{calc}</math><br/>(<math>\mu\text{g/mL}</math>)</b> | <b><math>NOAEC_{calc}</math><br/>(<math>\mu\text{g/mL}</math>)</b> | <b>ADI<br/>(<math>MIC_{calc}</math>),<sup>1</sup><br/><math>\mu\text{g/kg day}</math></b> | <b>ADI<br/>(<math>NOAEC_{calc}</math>),<sup>2</sup><br/><math>\mu\text{g/kg day}</math></b> |
|-------------------|--|--|---|---|
| amoxicillin       | 0.3  | 0.002  | 2.16  | 0.016   |
| cephalexin        | NA   | 0.07   | NA  | 2.182   |
| ciprofloxacin     | 0.2  | 0.001  | 1.15  | 0.006   |
| levofloxacin      | 0.1  | 0.002  | 2.24  | 0.043   |
| moxifloxacin      | 0.1  | 0.002  | 0.39  | 0.008   |
| doxycycline       | 0.2  | 0.01   | 0.66  | 0.028   |
| clindamycin       | 0.03   | 0.002  | 0.1   | 0.008   |
| metronidazole     | 0.1  | 0.01   | 0.84  | 0.068   |
| azithromycin      | 0.06   | 0.001  | 0.2   | 0.003   |
| sulfamethoxazole  | NA   | 0.002  | NA  | 0.036   |
| trimethoprim      | NA   | 0.2  | NA  | 1.842   |

ADI - acceptable daily intake, i.e., ADI for antibiotic intake via drinking water and fish; NA - not calculated because no MIC data was available for the set of 10 bacterial genera used in estimating  $MIC_{calc}$

<sup>1</sup> $MIC_{calc}$  were used to calculate the ADI values; <sup>2</sup> $NOAEC_{calc}$  were used to calculate the ADI values.

**Table S9.** Fractional oral doses of antibiotics available to intestinal microflora.

| <b>Antibiotic</b> | <b>Fraction of oral dose available to microorganisms (%)<sup>1</sup></b> |
|-------------------|--|
| amoxicillin       | 40   |
| cephalexin        | 10   |
| ciprofloxacin     | 60   |
| levofloxacin      | 13   |
| moxifloxacin      | 80   |
| doxycycline       | 99   |
| clindamycin       | 90   |
| metronidazole     | 40   |
| azithromycin      | 94   |
| sulfamethoxazole  | 16   |
| trimethoprim      | 33   |

<sup>1</sup>Calculated by subtracting the percentage excreted in urine, as reported in drug information sheets registered with the FDA, from 100.

**Table S10.** Ratios of predicted environmental concentrations (PEC, 99th percentile values) and predicted no effect concentrations (PNEC) which were at or above one for Columbia (C) and Sacramento (S) River watersheds (red values from Tables A1 and A2, respectively).

| Frame-<br>work            | River<br>flow             | Assumed<br>loss | River     | amoxi-<br>cillin | cepha-<br>lexin | cipro-<br>floxacin | levo-<br>floxacin | moxi-<br>floxacin | doxy-<br>cycline | clinda-<br>mycin | metro-<br>nidazole | azithro-<br>mycin | sulfa-<br>methoxa-<br>zole | trimetho-<br>prim |   |
|---------------------------|---------------------------|-----------------|-----------|------------------|-----------------|--------------------|-------------------|-------------------|------------------|------------------|--------------------|-------------------|----------------------------|-------------------|---|
| Larsson<br>frame-<br>work | Mean<br>flow              | All             | C         |                  |                 |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 | S         |                  |                 |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           | In stream       | C         | 3                |                 |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           | Low<br>flow               | WWTP            | C         |                  |                 |                    |                   |                   |                  |                  |                    | 1                 |                            |                   |   |
|                           |                           |                 | S         |                  |                 |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           | None            | C         | 3                |                 |                    |                   |                   |                  |                  | 1                  |                   |                            |                   |   |
|                           | Le Page<br>frame-<br>work | Mean<br>flow    | All       | C                | 1.5             |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 |           | S                | 1               |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 | In stream | C                | 37              |                    |                   | 2                 |                  |                  |                    |                   |                            |                   | 1 |
|                           |                           | Low<br>flow     | WWTP      | C                | 31              |                    | 3                 | 2                 |                  |                  |                    | 2                 |                            |                   |   |
|                           |                           |                 |           | S                | 2               |                    | 8                 | 1.4               |                  |                  | 9                  |                   |                            | 1                 |   |
|                           |                           |                 | None      | C                | 46              |                    | 25                | 3                 |                  |                  | 15                 |                   |                            | 2                 |   |
| Larsson<br>frame-<br>work |                           | Mean<br>flow    | All       | C                |                 |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 |           | S                |                 |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 | In stream | C                | 7               | 1                  |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           | Low<br>flow     | WWTP      | C                | 12              |                    |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 |           | S                |                 | 1                  |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 | None      | C                | 6               | 4                  |                   |                   |                  |                  | 1                  |                   |                            |                   |   |
|                           | Le Page<br>frame-<br>work | Mean<br>flow    | All       | C                | 13              | 5                  |                   |                   |                  |                  |                    | 2                 |                            | 1                 |   |
|                           |                           |                 |           | S                | 4               | 1                  |                   |                   |                  |                  |                    |                   |                            |                   |   |
|                           |                           |                 | In stream | C                | 3               | 1                  |                   | 2                 |                  |                  |                    |                   | 3                          | 4                 |   |
|                           |                           | Low<br>flow     | WWTP      | C                | 93              | 5                  |                   | 4                 |                  |                  | 1.4                |                   |                            | 5                 | 8 |
|                           |                           |                 |           | S                | 77              | 6                  |                   | 4                 |                  |                  | 1                  |                   | 2                          | 4                 | 6 |
|                           |                           |                 | None      | C                | 5               | 10                 | 1                 | 3                 |                  |                  | 1.4                | 1.6               | 12                         | 3                 | 5 |
| Larsson<br>frame-<br>work |                           | Mean<br>flow    | All       | C                | 4               | 1                  |                   | 2                 |                  |                  |                    |                   | 3                          | 4                 |   |
|                           |                           |                 |           | S                | 3               | 1                  |                   | 2                 |                  |                  |                    | 1                 | 2                          | 3                 |   |
|                           |                           |                 | In stream | C                | 93              | 5                  |                   | 4                 |                  |                  | 1.4                |                   |                            | 5                 | 8 |
|                           |                           | Low<br>flow     | WWTP      | C                | 77              | 6                  |                   | 4                 |                  |                  | 1                  |                   | 2                          | 4                 | 6 |
|                           |                           |                 |           | S                | 5               | 10                 | 1                 | 3                 |                  |                  | 1.4                | 1.6               | 12                         | 3                 | 5 |
|                           |                           |                 | None      | C                | 4               | 7                  |                   | 3                 |                  |                  |                    | 1                 | 8                          | 2                 | 3 |
|                           | Le Page<br>frame-<br>work | Mean<br>flow    | All       | C                | 114             | 54                 | 4                 | 8                 |                  | 4                | 1.6                | 19                | 5                          | 9                 |   |
|                           |                           |                 |           | S                | 90              | 36                 | 3                 | 6                 |                  | 3                | 1                  | 13                | 4                          | 7                 |   |
|                           |                           |                 | In stream | C                | 93              | 5                  |                   | 4                 |                  |                  | 1.4                |                   |                            | 5                 | 8 |
|                           |                           | Low<br>flow     | WWTP      | C                | 77              | 6                  |                   | 4                 |                  |                  | 1                  |                   | 2                          | 4                 | 6 |
|                           |                           |                 |           | S                | 5               | 10                 | 1                 | 3                 |                  |                  | 1.4                | 1.6               | 12                         | 3                 | 5 |
|                           |                           |                 | None      | C                | 4               | 7                  |                   | 3                 |                  |                  |                    | 1                 | 8                          | 2                 | 3 |

|                                 |                      |                  |          |    |   |    |     |   |
|---------------------------------|----------------------|------------------|----------|----|---|----|-----|---|
| <b>VICH<br/>frame-<br/>work</b> | <b>Mean<br/>flow</b> | <b>All</b>       | <b>C</b> |    |   |    |     |   |
|                                 |                      |                  | <b>S</b> |    |   |    |     |   |
|                                 |                      | <b>In stream</b> | <b>C</b> | 1  |   |    |     |   |
|                                 |                      |                  | <b>S</b> | 2  |   |    | 1   |   |
|                                 |                      | <b>WWTP</b>      | <b>C</b> |    |   |    |     |   |
|                                 |                      |                  | <b>S</b> |    |   |    |     |   |
|                                 |                      | <b>None</b>      | <b>C</b> | 1  |   |    |     |   |
|                                 |                      |                  | <b>S</b> | 2  |   |    | 1   |   |
|                                 |                      | <b>All</b>       | <b>C</b> |    |   |    | 6   | 2 |
|                                 |                      |                  | <b>S</b> |    |   |    | 4   | 1 |
|                                 | <b>Low<br/>flow</b>  | <b>In stream</b> | <b>C</b> | 17 |   |    | 10  | 4 |
|                                 |                      |                  | <b>S</b> | 14 | 1 |    | 7   | 3 |
|                                 | <b>WWTP</b>          | <b>C</b>         |          | 2  |   | 6  | 2   |   |
|                                 |                      | <b>S</b>         |          | 2  |   | 4  | 1.5 |   |
|                                 | <b>None</b>          | <b>C</b>         | 21       | 8  |   | 11 | 5   |   |
|                                 |                      | <b>S</b>         | 16       | 6  |   | 8  | 3   |   |



<sup>a</sup>Kolpin et al. (2002): measured concentrations in U.S. streams; <sup>b</sup>Anderson et al. (2012): Table E1: Aqueous concentration values and data sources for occurrence metric and Los Angeles Regional Board (LARB) River Study maximum occurrence values, streams and effluents in the U.S.; <sup>c</sup>Anderson et al. (2012): Table E2: Aqueous concentrations (ng/L) utilized in hazard calculations for WERF CEC5R8a (Diamond et al., 2011), streams and effluents in the U.S.; <sup>d</sup>Verlicchi et al. (2012): review of literature data, WWTP secondary effluents from 78 WWTPs across the world; <sup>e</sup>Hughes et al. (2013): extensive literature review on concentrations in freshwater ecosystems of 41 countries, median values; <sup>f</sup>Hughes et al. (2013): maximum values; <sup>g</sup>Klosterhaus et al. (2013): urban estuary in California, the U.S.; <sup>h</sup>Rodriguez-Mozaz et al. (2015): one WWTP in Spain, WWTP effluent; <sup>i</sup>Rodriguez-Mozaz et al. (2015): downstream of WWTP; <sup>j</sup>Carvalho and Santos (2016): literature data on European environmental aqueous matrices, means of reported values were calculated for this study, river water; <sup>k</sup>Carvalho and Santos (2016): WWTP effluents; <sup>l</sup>Lautz et al. (2017): literature data, WWTP effluents in Europe; <sup>m</sup>Grenni et al. (2018): literature data on concentrations in three Italian Rivers, maximum reported concentrations were included in the table; <sup>n</sup>Bexfield et al. (2019): 1114 wells and six springs sampled in the U.S. and measured for 21 hormones and 105 pharmaceuticals, groundwater. NR – not reported or excluded from the current analysis if the value was orders of magnitude higher than the rest of the collected values; ND – not detected.

**Calculation of antibiotic sales in kilograms (kg) in Sacramento and Columbia River watershed areas in 2011.**

To estimate the antibiotic usage (kg/year) for the Sacramento River watershed (**Table S3**), the national sales data (**Table 1**) was adjusted by the ratio of California prescription rate to national prescription rate (**Table S2**) as follows:

$$Usage(Sacramento) = \frac{Prescriptions_{California}}{Prescriptions_{national}} \times Sales_{national}$$

where:  $Prescriptions_{California}$  = antibiotic prescription rate (number of prescriptions per 1000 persons) in California

$Prescriptions_{national}$  = antibiotic prescription rate (number of prescriptions per 1000 persons) at national level

$Sales_{national}$  = national sales of the antibiotic (kg/year)

To estimate the antibiotic usage (kg/year) for the Columbia River watershed (**Table S3**), the national sales data (**Table 1**) was adjusted by the ratio of each of the four state's prescription rate to national prescription rate (**Table S2**) and weighted by each state's population (**Table S4**). Population for the four States in the Columbia River watershed was obtained from 2010 government censuses (U.S. Census Bureau, 2012).

$$Usage(Columbia) = \frac{\sum_{4\ states} \left( \frac{Prescriptions_{state}}{Prescriptions_{national}} \times Population_{state} \right)}{Population_{4\ states}} \times Sales_{national}$$

where:  $Prescriptions_{state}$  = antibiotic prescription rate (number of prescriptions per 1000 persons) in each of the 4 states of Columbia River watershed

$Prescriptions_{national}$  = antibiotic prescription rate (number of prescriptions per 1000 persons) at national level

$Population_{state}$  = population of each of the 4 states of Columbia River watershed

$Population_{4\ states}$  = total population of the 4 states of Columbia River watershed

$Sales_{national}$  = national sales of the antibiotic (kg/year)

**Calculation of highest acceptable human drinking water concentrations (HDWC) based on the guidance issued by FDA's Center for Veterinary Medicine (VICH, 2012).**

The lower 90% confidence limit (CL) of means of log-transformed  $MIC_{min}$  or  $MIC_{50}$  values was calculated using the following formula:

$$Lower\ 90\%\ CL = mean(MIC_{min}\ or\ MIC_{50}) - \frac{StdDev(MIC_{min}\ or\ MIC_{50})}{\sqrt{n}} \times t_{0.10,df}$$

where:  $mean(MIC_{min}\ or\ MIC_{50})$  = the mean of the log-transformed  $MIC_{min}$  or  $MIC_{50}$  values

$StdDev(MIC_{min}\ or\ MIC_{50})$  = the standard deviation of the log-transformed  $MIC_{min}$  or  $MIC_{50}$  values

$n$  = the number of  $MIC_{min}$  or  $MIC_{50}$  values used in the calculations

$t_{0.10,df}$  = the 90<sup>th</sup> percentile from the central t-distribution with n-1 degrees of freedom (df)

Then the  $NOAEC_{calc}$  and  $MIC_{calc}$  (**Table S8**) were derived as follows:

$$NOAEC_{calc}\ or\ MIC_{calc} = 2^{[Lower\ 90\%\ CL + Log_2((MIC_{min}\ or\ MIC_{50})/2)]}$$

where:  $MIC_{min}\ or\ MIC_{50}$  = the lowest  $MIC_{min}$  or  $MIC_{50}$  value for all strains (untransformed)

A microbiological ADI ( $\mu\text{g}/\text{kg}$ ) for an antibiotic was calculated using either  $\text{MIC}_{\text{calc}}$  or  $\text{NOAEC}_{\text{calc}}$ , whichever was lower, as follows:

$$\text{Microbiological ADI} = \frac{(\text{MIC}_{\text{calc}} \text{ or } \text{NOAEC}_{\text{calc}}) \times \text{Mass}_{\text{colon}}}{\% \text{ available} \times \text{body weight}}$$

where:  $\text{MIC}_{\text{calc}}$  = the lower 90% confidence interval for the mean  $\text{MIC}_{50}$  of relevant human gut genera for which the drug is active (protects for disruption of the colonization barrier)

$\text{NOAEC}_{\text{calc}}$  = the lower 90% confidence interval for the mean  $\text{MIC}_{\text{min}}$  from in vitro systems (reduces the potential for resistance selection in label indications)

$\text{Mass}_{\text{colon}}$  = 220g, based on the colon content measured from human accident victims

$\% \text{ available}$  = the fraction of oral dose available for intestinal microorganisms (**Table S9**)

$\text{body weight}$  = body weight of adult humans, assumed to be 70 kg

The microbiological ADI ( $\mu\text{g}/\text{kg}$ ) was used to calculate HDWC (**Table 3**) using the principles of US EPA Water Quality Criteria Guidance document (EPA, 2000) by assuming that humans could be exposed to an antibiotic via drinking 2 L of water (for an adult) as well as eating an average of 17.5 g of fish from water near the drinking water intake:

$$\text{HDWC} = \frac{\text{Mg}T_{\text{human}}}{V_{\text{water}} + (\text{Consumption}_{\text{fish}} \times \text{BCF})}$$

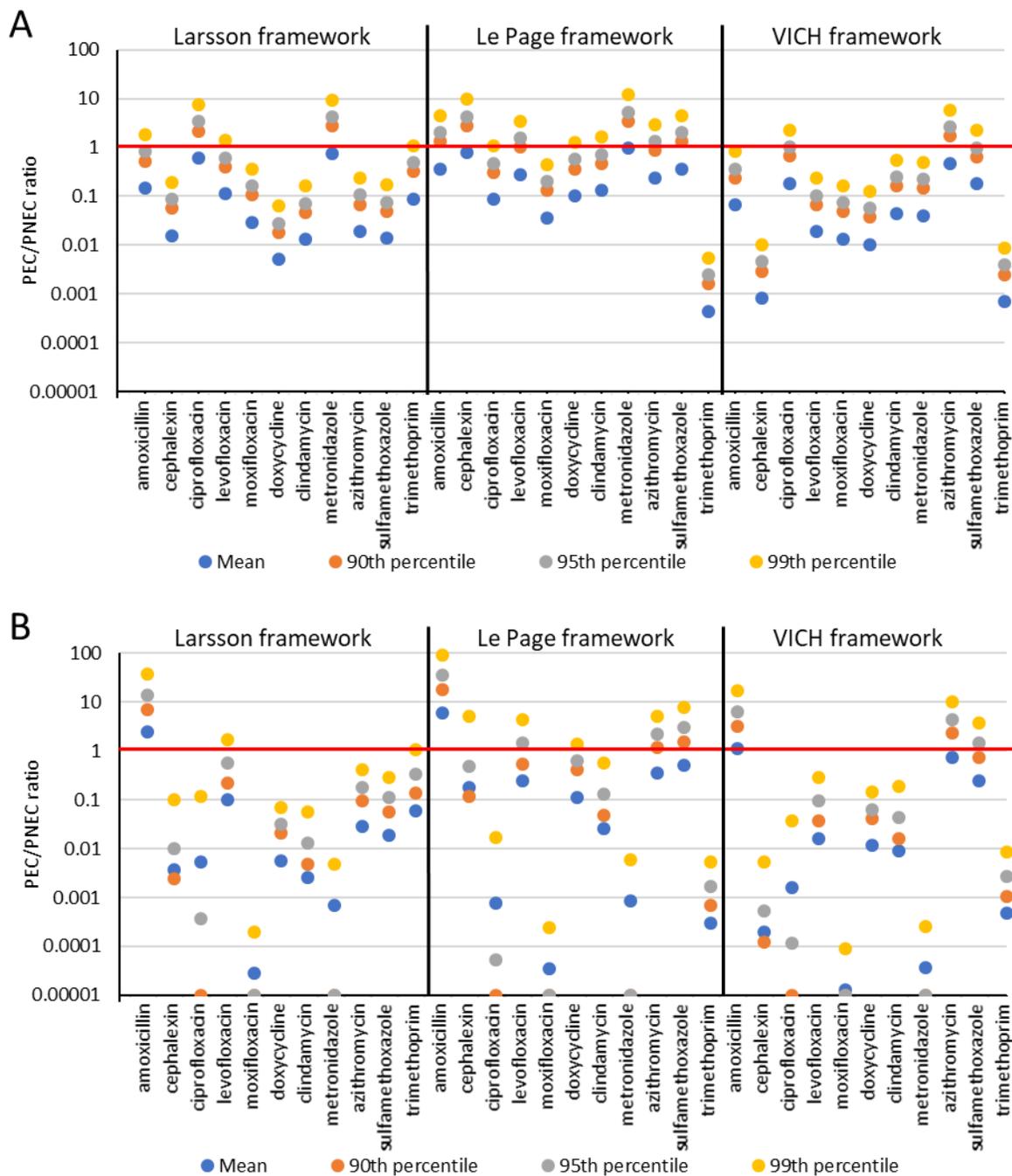
where:  $\text{Mg}T_{\text{human}}$  = ADI for 70 kg person

$V_{\text{water}}$  = 2 L/day (average for adult)

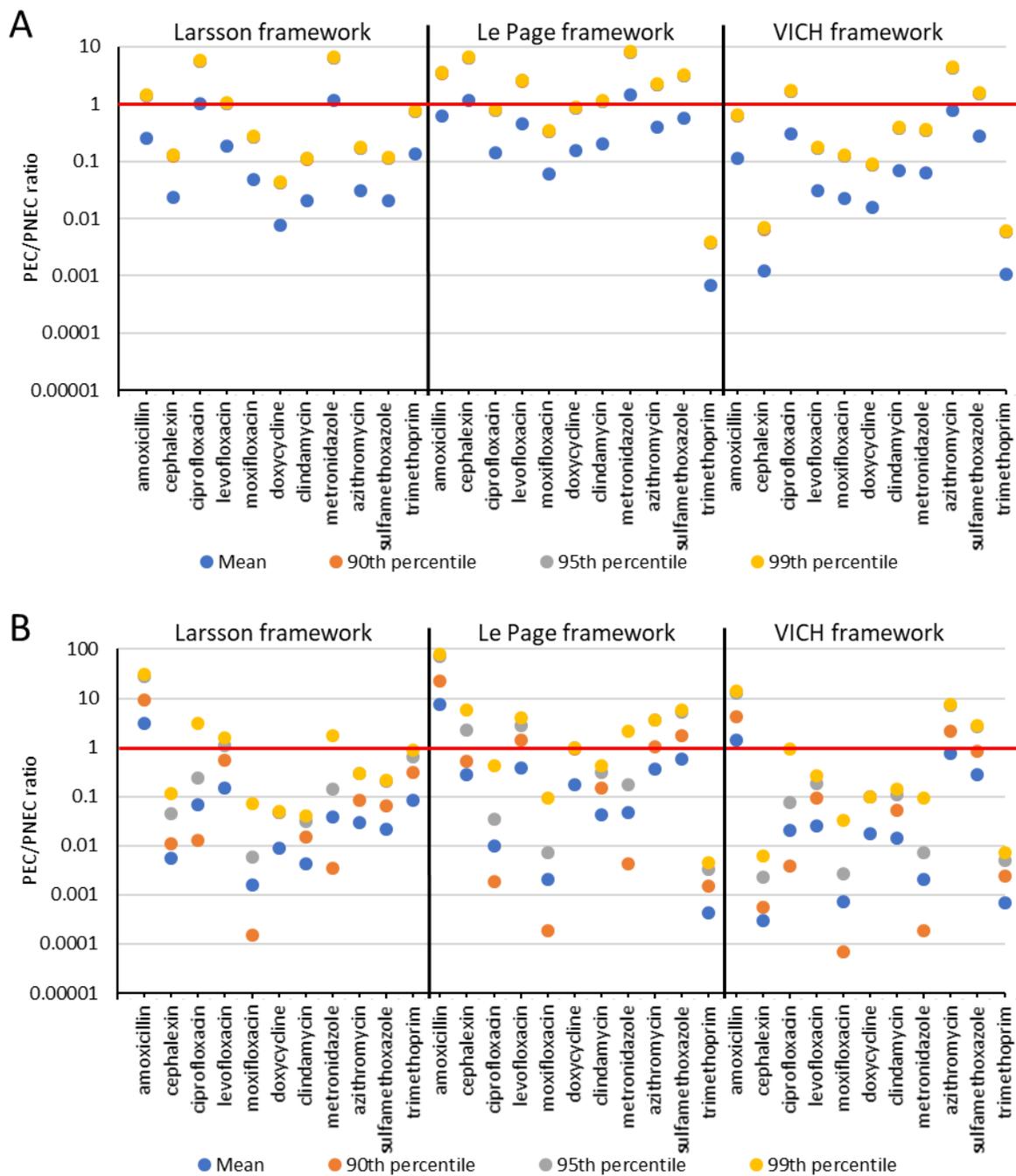
$\text{Consumption}_{\text{fish}}$  = 0.0175 kg/day (average for adult)

$\text{BCF}$  = bioconcentration factor (in fish)

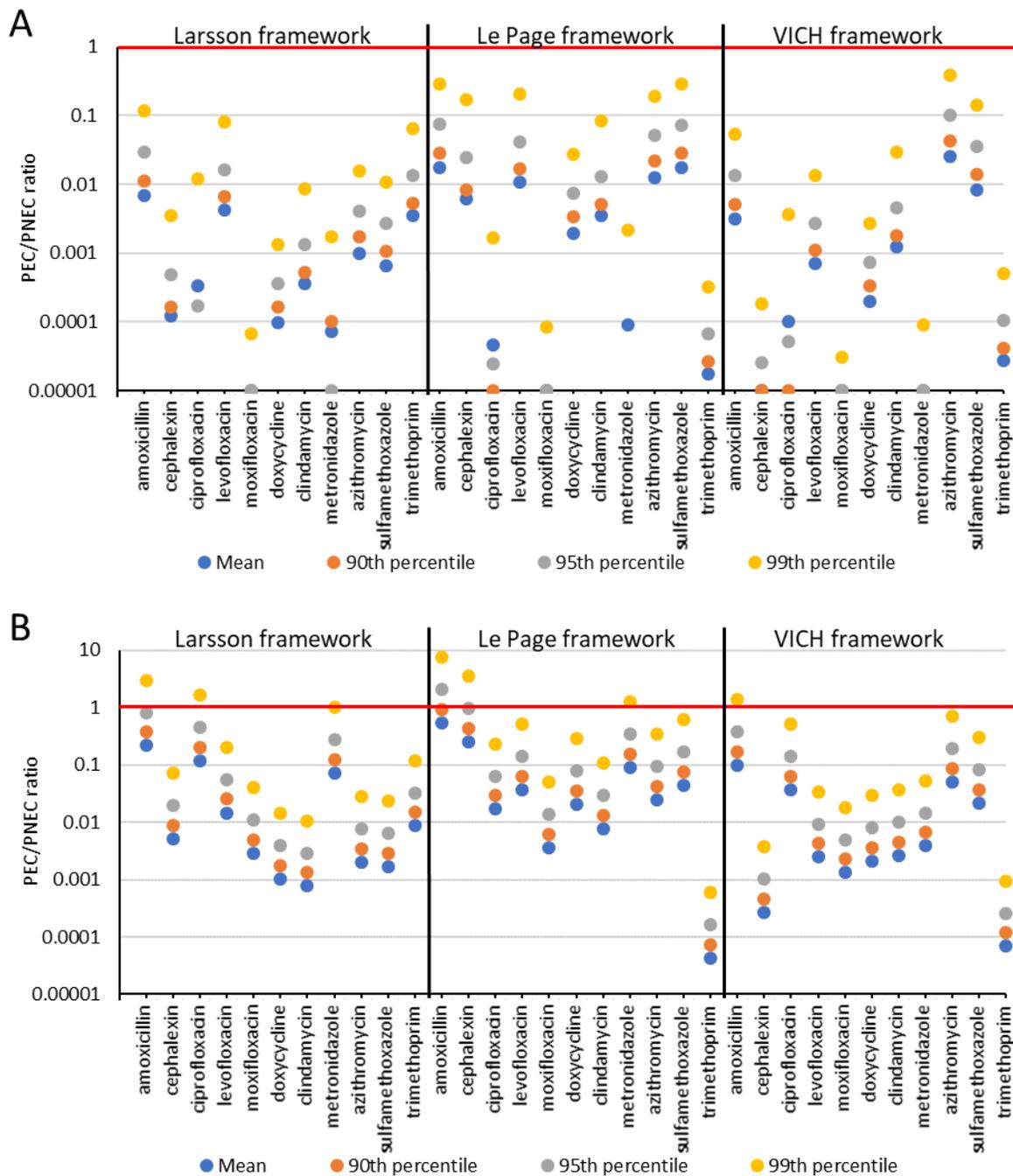
BCF was estimated based on a model by Veith et al. (1979) recommended for substances with  $\log K_{ow}$  between 0 and 6 (**Table S1**; Muller and Nendza, 2011) and was corrected for fish lipid content.



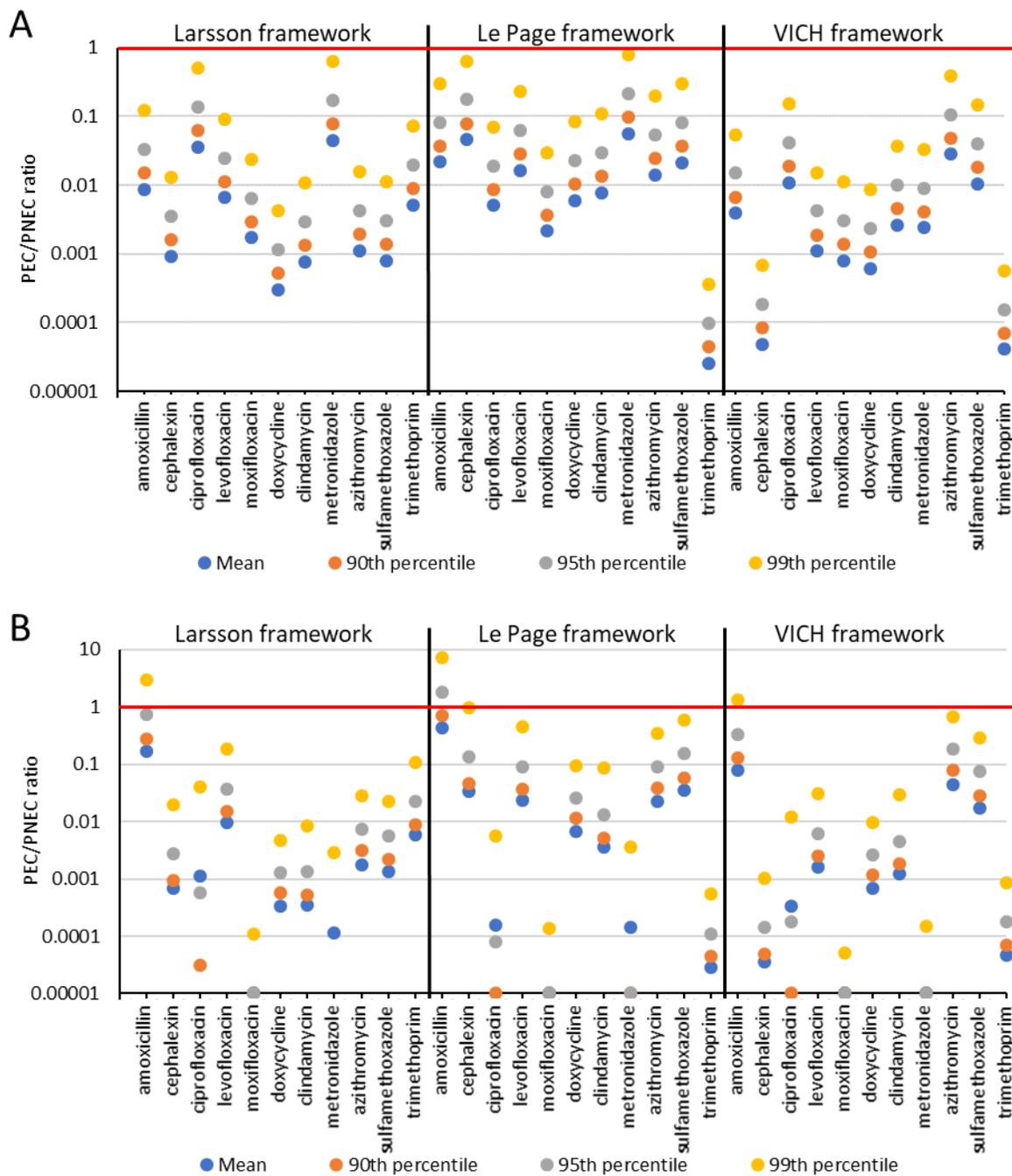
**Figure S1.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Columbia River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming low river flow and accounting for antibiotic losses due to (A) human metabolism and wastewater treatment plant removal, i.e. assumed loss: “WWTP”, or (B) human metabolism and in stream degradation i.e. assumed loss: “In-stream”. Mean, 90<sup>th</sup>, 95<sup>th</sup> and 99<sup>th</sup> percentile PEC of 750 segments of the Columbia River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one.



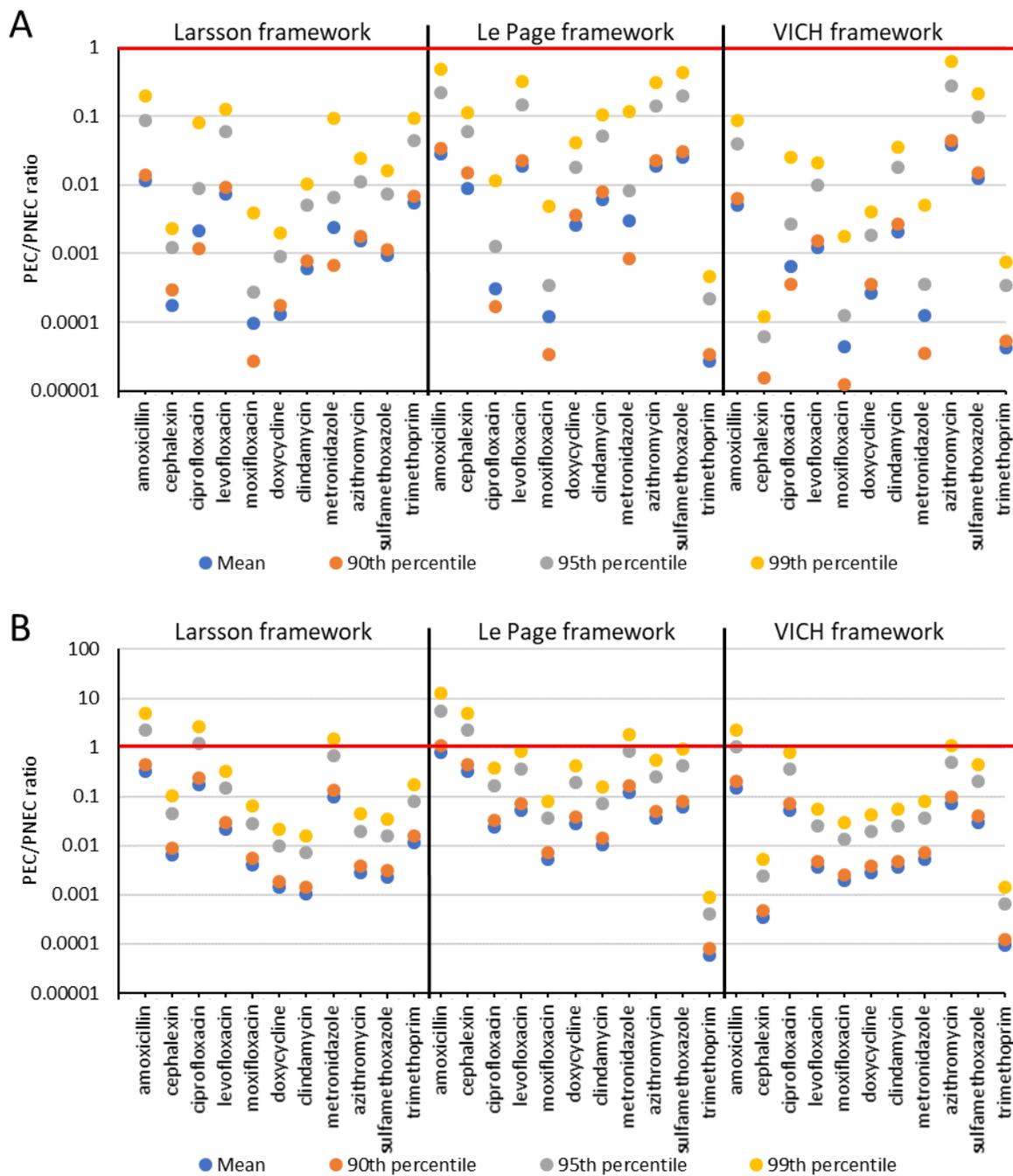
**Figure S2.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Sacramento River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming low river flow and accounting for antibiotic losses due to (A) human metabolism and wastewater treatment plant removal, i.e. assumed loss: “WWTP”, or (B) human metabolism and in stream degradation i.e. assumed loss: “In-stream”. Mean, 90<sup>th</sup>, 95<sup>th</sup> and 99<sup>th</sup> percentile PEC of 55 segments of the Sacramento River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one. In panel A, yellow data points overlap with orange and grey data points.



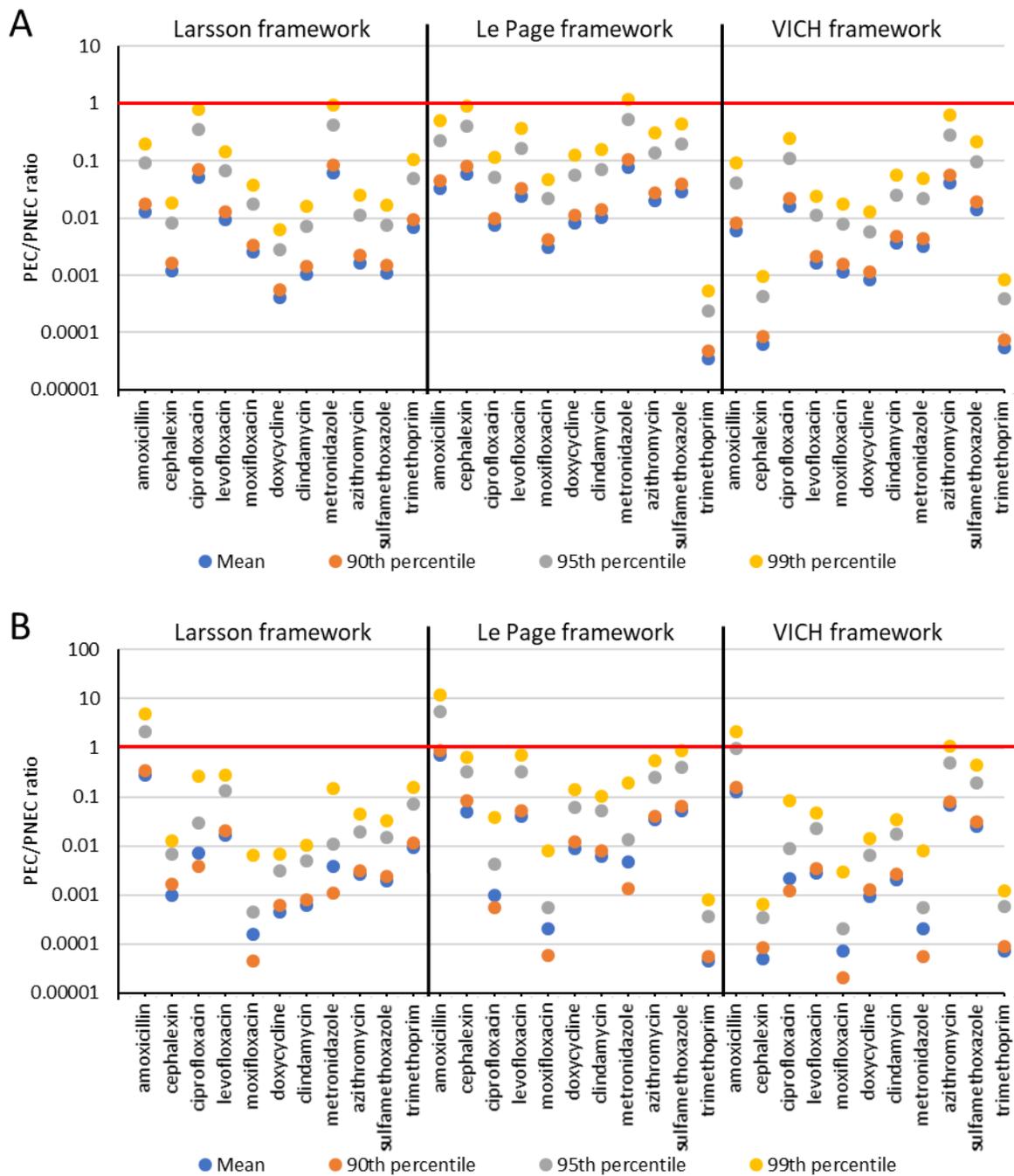
**Figure S3.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Columbia River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming mean river flow and accounting for antibiotic losses due to (A) human metabolism, in stream degradation and wastewater treatment plant removal, i.e. assumed loss: “All”, or (B) only human metabolism, i.e. assumed loss: “None”. Mean, 90<sup>th</sup>, 95<sup>th</sup> and 99<sup>th</sup> percentile PEC of 750 segments of the Columbia River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one.



**Figure S4.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Columbia River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming mean river flow and accounting for antibiotic losses due to (A) human metabolism and wastewater treatment plant removal, i.e. assumed loss: “WWTP”, or (B) human metabolism and in stream degradation i.e. assumed loss: “In-stream”. Mean, 90<sup>th</sup>, 95<sup>th</sup> and 99<sup>th</sup> percentile PEC of 750 segments of the Columbia River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one.



**Figure S5.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Sacramento River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming mean river flow and accounting for antibiotic losses due to (A) human metabolism, in stream degradation and wastewater treatment plant removal, i.e. assumed loss: “All”, or (B) only human metabolism, i.e. assumed loss: “None”. Mean, 90<sup>th</sup>, 95<sup>th</sup> and 99<sup>th</sup> percentile PEC of 55 segments of the Sacramento River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one.



**Figure S6.** Ratios of antibiotic predicted environmental concentrations (PEC) in the Sacramento River watershed and predicted no effect concentrations (PNEC) of the Larsson, Le Page and VICH frameworks. PEC values were simulated using *PhATE*, assuming mean river flow and accounting for antibiotic losses due to (A) human metabolism and wastewater treatment plant removal, i.e. assumed loss: “WWTP”, or (B) human metabolism and in stream degradation i.e. assumed loss: “In-stream”. Mean, 90<sup>th</sup>, 95<sup>th</sup> and 99<sup>th</sup> percentile PEC of 55 segments of the Sacramento River watershed were used in PEC/PNEC ratio calculations. Red lines mark PEC/PNEC ratio equal to one.

## References

- Anderson, P., Denslow, N., Drewes, J., livieri, A., Schlenk, D., Scott, G., Snyder, S., 2012. Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems. Southern California Coastal Water Research Project. Technical Report. [https://www.waterboards.ca.gov/water\\_issues/programs/swamp/cec\\_aquatic/docs/cec\\_ecosystems\\_rpt.pdf](https://www.waterboards.ca.gov/water_issues/programs/swamp/cec_aquatic/docs/cec_ecosystems_rpt.pdf).
- Aucamp, M., Odendaal, R., Liebenberg, W., Hamman, J., 2015. Amorphous azithromycin with improved aqueous solubility and intestinal membrane permeability. *Drug Development and Industrial Pharmacy* 41, 1100-1108. DOI: 10.3109/03639045.2014.931967.
- Bexfield, L.M., Toccalino, P.L., Belitz, K., Foreman, W.T., Furlong, E.T., 2019. Hormones and pharmaceuticals in groundwater used as a source of drinking water across the United States. *Environmental Science & Technology* 53, 2950-2960. DOI: 10.1021/acs.est.8b05592.
- Braschi, I., Blasioli, S., Fellet, C., Lorenzini, R., Garelli, A., Pori, M., Giacomini, D., 2013. Persistence and degradation of new  $\beta$ -lactam antibiotics in the soil and water environment. *Chemosphere* 93, 152-159. DOI: <https://doi.org/10.1016/j.chemosphere.2013.05.016>.
- Carvalho, I.T., Santos, L., 2016. Antibiotics in the aquatic environments: A review of the European scenario. *Environment International* 94, 736-757. DOI: <https://doi.org/10.1016/j.envint.2016.06.025>.
- CDC, 2011. Centers for Disease Control and Prevention. Antibiotic Prescription Rates at National and State Levels in 2011. <https://gis.cdc.gov/grasp/PSA/AUMapView.html>. Accessed: July, 2018.
- EPA, 2000. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000). EPA-822-B-00-004.
- Erah, P.O., Goddard, A.F., Barrett, D.A., Shaw, P.N., Spiller, R.C., 1997. The stability of amoxicillin, clarithromycin and metronidazole in gastric juice: relevance to the treatment of *Helicobacter pylori* infection. *The Journal of Antimicrobial Chemotherapy* 39, 5-12. DOI: 10.1093/jac/39.1.5.
- Felix, I.M.B., Moreira, L.C., Chiavone-Filho, O., Mattedi, S., 2016. Solubility measurements of amoxicillin in mixtures of water and ethanol from 283.15 to 298.15 K. *Fluid Phase Equilibria* 422, 78-86. DOI: <https://doi.org/10.1016/j.fluid.2016.02.040>.

Gonzalez, J.A., Callejon Mochon, M., de la Rosa, F.J., 2000. Spectrofluorimetric determination of levofloxacin in tablets, human urine and serum. *Talanta* 52, 1149-1156.

Grenni, P., Ancona, V., Barra Caracciolo, A., 2018. Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal* 136, 25-39. DOI: <https://doi.org/10.1016/j.microc.2017.02.006>.

Henzler, A.F., Greskowiak, J., Massmann, G., 2014. Modeling the fate of organic micropollutants during river bank filtration (Berlin, Germany). *Journal of Contaminant Hydrology* 156, 78-92. DOI: <https://doi.org/10.1016/j.jconhyd.2013.10.005>.

Hirano, T., Yasuda, S., Osaka, Y., Kobayashi, M., Itagaki, S., Iseki, K., 2006. Mechanism of the inhibitory effect of zwitterionic drugs (levofloxacin and grepafloxacin) on carnitine transporter (OCTN2) in Caco-2 cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1758, 1743-1750. DOI: <https://doi.org/10.1016/j.bbamem.2006.07.002>.

HSDB, 2018. Hazardous Substances Data Bank, <https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm>, Accessed: July 2018.

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. *Environmental Science & Technology* 47, 661-677. DOI: 10.1021/es3030148.

Im, H., Yeo, I., Maeng, S.K., Park, C.H., Choi, H., 2016. Simultaneous attenuation of pharmaceuticals, organic matter, and nutrients in wastewater effluent through managed aquifer recharge: Batch and column studies. *Chemosphere* 143, 135-141. DOI: [10.1016/j.chemosphere.2015.10.104](https://doi.org/10.1016/j.chemosphere.2015.10.104).

Jia, A., Wan, Y., Xiao, Y., Hu, J., 2012. Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. *Water Research* 46, 387-394. DOI: <https://doi.org/10.1016/j.watres.2011.10.055>.

Jin, J., Feng, T., Gao, R., Ma, Y., Wang, W., Zhou, Q., Li, A., 2018. Ultrahigh selective adsorption of zwitterionic PPCPs both in the absence and presence of humic acid: Performance and mechanism. *Journal of Hazardous Materials* 348, 117-124. DOI: [10.1016/j.jhazmat.2018.01.036](https://doi.org/10.1016/j.jhazmat.2018.01.036).

Jjemba, P.K., 2006. Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. *Ecotoxicology and Environmental Safety* 63, 113-130. DOI: <https://doi.org/10.1016/j.ecoenv.2004.11.011>.

Kansy, M., Fischer, H., Kratzat, K., Senner, F., Wagner, B. and Parrilla, I., 2007. High-Throughput Artificial Membrane Permeability Studies in Early Lead Discovery and Development. *Pharmacokinetic Optimization in Drug Research*. H.v.d.W. B. Testa, G. Folkers and R. Guy: 447-464.

Kimura, K., Toshima, S., Amy, G., Watanabe, Y., 2004. Rejection of neutral endocrine disrupting compounds (EDCs) and pharmaceutical active compounds (PhACs) by RO membranes. *Journal of Membrane Science* 245, 71-78. DOI: <https://doi.org/10.1016/j.memsci.2004.07.018>.

Klempner, M.S., Styrt, B., 1983. Alkalinization of the intralysosomal pH by clindamycin and its effects on neutrophil function. *The Journal of Antimicrobial Chemotherapy* 12 Suppl C, 39-50. DOI: 10.1093/jac/12.suppl\_c.39.

Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. *Environment International* 54, 92-99. DOI: <https://doi.org/10.1016/j.envint.2013.01.009>.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environmental Science & Technology* 36, 1202-1211. DOI: 10.1021/es011055j.

Lam, M.W., Young, C.J., Brain, R.A., Johnson, D.J., Hanson, M.A., Wilson, C.J., Richards, S.M., Solomon, K.R., Mabury, S.A., 2004. Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environmental Toxicology and Chemistry* 23, 1431-1440. DOI: 10.1897/03-421.

Langlois, M.-H., Montagut, M., Dubost, J.-P., Grellet, J., Saux, M.-C., 2005. Protonation equilibrium and lipophilicity of moxifloxacin. *Journal of Pharmaceutical and Biomedical Analysis* 37, 389-393. DOI: <https://doi.org/10.1016/j.jpba.2004.10.022>.

Lautz, L.S., Struijs, J., Nolte, T.M., Breure, A.M., van der Grinten, E., van de Meent, D., van Zelm, R., 2017. Evaluation of SimpleTreat 4.0: Simulations of pharmaceutical removal in wastewater treatment plant facilities. *Chemosphere* 168, 870-876. DOI: 10.1016/j.chemosphere.2016.10.123.

Legendre, A.O., Silva, L.R.R., Silva, D.M., Rosa, I.M.L., Azarias, L.C., de Abreu, P.J., de Araújo, M.B., Neves, P.P., Torres, C., Martins, F.T., Doriguetto, A.C., 2012. Solid state chemistry of the antibiotic doxycycline: structure of the neutral monohydrate and insights into its poor water solubility. *CrystEngComm* 14, 2532-2540. DOI: 10.1039/C1CE06181J.

Lombardo, F., Shalaeva, M.Y., Tupper, K.A., Gao, F., Abraham, M.H., 2000. ElogPoct: a tool for lipophilicity determination in drug discovery. *Journal of Medicinal Chemistry* 43, 2922-2928. DOI: 10.1021/jm0000822.

Matsumoto, S., Saito, H., Inui, K., 1994. Transcellular transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: interaction with dipeptide transport systems in apical and basolateral membranes. *The Journal of Pharmacology and Experimental Therapeutics* 270, 498-504.

McFarland, J.W., Berger, C.M., Froshauer, S.A., Hayashi, S.F., Hecker, S.J., Jaynes, B.H., Jefson, M.R., Kamicker, B.J., Lipinski, C.A., Lundy, K.M., Reese, C.P., Vu, C.B., 1997. Quantitative structure-activity relationships among macrolide antibacterial agents: in vitro and in vivo potency against *Pasteurella multocida*. *Journal of Medicinal Chemistry* 40, 1340-1346. DOI: 10.1021/jm960436i.

Muller, M., Nendza, M., 2011. Comparative analysis of estimated and measured BCF data (OECD 305). Umweltbundesamt, Germany.

Obach, R.S., Lombardo, F., Waters, N.J., 2008. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 36, 1385-1405. DOI: 10.1124/dmd.108.020479.

Park, J., Yamashita, N., Park, C., Shimono, T., Takeuchi, D.M., Tanaka, H., 2017. Removal characteristics of pharmaceuticals and personal care products: Comparison between membrane bioreactor and various biological treatment processes. *Chemosphere* 179, 347-358. DOI: <https://doi.org/10.1016/j.chemosphere.2017.03.135>.

Qiang, Z., Adams, C., 2004. Potentiometric determination of acid dissociation constants (pKa) for human and veterinary antibiotics. *Water Research* 38, 2874-2890. DOI: <https://doi.org/10.1016/j.watres.2004.03.017>.

Quiming, N.S., Denola, N.L., Saito, Y., Ueta, I., Ogawa, M., Jinno, K., 2007. Effect of counter-anions on the retention of zwitterionic quinolones in reversed-phase liquid chromatography. *Journal of Liquid Chromatography & Related Technologies* 30, 1343-1360. DOI: 10.1080/10826070701276465.

Rafols, C., Subirats, X., Rubio, J., Roses, M., Bosch, E., 2017. Lipophilicity of amphoteric and zwitterionic compounds: A comparative study of determination methods. *Talanta* 162, 293-299. DOI: 10.1016/j.talanta.2016.10.038.

Refaat, H.M., El-Badway, H.A., Morgan, S.M., 2016. Molecular docking, geometrical structure, potentiometric and thermodynamic studies of moxifloxacin and its metal complexes. *Journal of Molecular Liquids* 220, 802-812. DOI: <https://doi.org/10.1016/j.molliq.2016.04.124>.

Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sanchez-Melsio, A., Borrego, C.M., Barcelo, D., Balcazar, J.L., 2015. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Research* 69, 234-242. DOI: 10.1016/j.watres.2014.11.021.

Shalaeva, M., Kenseth, J., Lombardo, F., Bastin, A., 2008. Measurement of dissociation constants (pKa values) of organic compounds by multiplexed capillary electrophoresis using aqueous and cosolvent buffers. *Journal of Pharmaceutical Sciences* 97, 2581-2606. DOI: 10.1002/jps.21287.

Sturini, M., Speltini, A., Maraschi, F., Profumo, A., Pretali, L., Irastorza, E.A., Fasani, E., Albini, A., 2012. Photolytic and photocatalytic degradation of fluoroquinolones in untreated river water under natural sunlight. *Applied Catalysis B: Environmental* 119-120, 32-39. DOI: <https://doi.org/10.1016/j.apcatb.2012.02.008>.

Takács-Novák, K., Józán, M., Hermecz, I., Szász, G., 1992. Lipophilicity of antibacterial fluoroquinolones. *International Journal of Pharmaceutics* 79, 89-96. DOI: [https://doi.org/10.1016/0378-5173\(92\)90099-N](https://doi.org/10.1016/0378-5173(92)90099-N).

Tong, L., Perez, S., Goncalves, C., Alpendurada, F., Wang, Y., Barcelo, D., 2011. Kinetic and mechanistic studies of the photolysis of metronidazole in simulated aqueous environmental matrices using a mass spectrometric approach. *Analytical and Bioanalytical Chemistry* 399, 421-428. DOI: 10.1007/s00216-010-4320-5.

Varanda, F., Pratas de Melo, M.J., Caço, A.I., Dohrn, R., Makrydaki, F.A., Voutsas, E., Tassios, D., Marrucho, I.M., 2006. Solubility of antibiotics in different solvents. 1. Hydrochloride forms of tetracycline, moxifloxacin, and ciprofloxacin. *Industrial & Engineering Chemistry Research* 45, 6368-6374. DOI: 10.1021/ie060055v.

Veith, G.D., DeFoe, D.L., Bergstedt, B.V., 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. *Journal of the Fisheries Research Board of Canada* 36, 1040-1048. DOI: 10.1139/f79-146.

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of the Total Environment* 429, 123-155. DOI: <https://doi.org/10.1016/j.scitotenv.2012.04.028>.

Vermillion Maier, M.L., Tjeerdema, R.S., 2018. Azithromycin sorption and biodegradation in a simulated California river system. *Chemosphere* 190, 471-480. DOI: <https://doi.org/10.1016/j.chemosphere.2017.10.008>.

VICH, 2012. VICH GL36(R) Guidance for Industry. Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine: 2012.

Viswanadhan, V.N., Ghose, A.K., Revankar, G.R., Robins, R.K., 1989. Atomic physicochemical parameters for three dimensional structure directed quantitative structure-activity relationships. 4. Additional parameters for hydrophobic and dispersive interactions and their application for an automated superposition of certain naturally occurring nucleoside antibiotics. *Journal of Chemical Information and Computer Sciences* 29, 163-172. DOI: 10.1021/ci00063a006.

Wan, H., Holmén, A.G., Wang, Y., Lindberg, W., Englund, M., Någård, M.B., Thompson, R.A., 2003. High-throughput screening of pKa values of pharmaceuticals by pressure-assisted capillary electrophoresis and mass spectrometry. *Rapid Communications in Mass Spectrometry* 17, 2639-2648. DOI: 10.1002/rcm.1229.

Wang, F., Feng, Y., Chen, P., Wang, Y., Su, Y., Zhang, Q., Zeng, Y., Xie, Z., Liu, H., Liu, Y., Lv, W., Liu, G., 2018. Photocatalytic degradation of fluoroquinolone antibiotics using ordered mesoporous g-C<sub>3</sub>N<sub>4</sub> under simulated sunlight irradiation: Kinetics, mechanism, and antibacterial activity elimination. *Applied Catalysis B: Environmental* 227, 114-122. DOI: <https://doi.org/10.1016/j.apcatb.2018.01.024>.

Winiwarter, S., Bonham, N.M., Ax, F., Hallberg, A., Lennernas, H., Karlen, A., 1998. Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *Journal of Medicinal Chemistry* 41, 4939-4949. DOI: 10.1021/jm9810102.

Yalkowsky, S.H., He, Y., 2003. Handbook of aqueous solubility data. Boca Raton, Fla., CRC Press.

Yan, S., Yao, B., Lian, L., Lu, X., Snyder, S.A., Li, R., Song, W., 2017. Development of fluorescence surrogates to predict the photochemical transformation of pharmaceuticals in wastewater effluents. *Environmental Science & Technology* 51, 2738-2747. DOI: 10.1021/acs.est.6b05251.

Zaranyika, M.F., Dzomba, P., Kugara, J., 2015. Speciation and persistence of doxycycline in the aquatic environment: Characterization in terms of steady state kinetics. *Journal of Environmental Science and Health, Part B* 50, 908-918. DOI: 10.1080/03601234.2015.1067101.

Zrnčić, M., Babić, S., Mutavdžić Pavlović, D., 2015. Determination of thermodynamic pKa values of pharmaceuticals from five different groups using capillary electrophoresis. *Journal of Separation Science* 38, 1232-1239. DOI: 10.1002/jssc.201401057.