UNIVERSITÄT OSNABRÜCK

Geography-referenced modeling of pharmaceuticals and fecal bacteria for risk assessment in river catchments

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Dissertation
for the degree of Doctor of Natural Sciences (Dr.rer.nat.)

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Osnabrück, March 2023

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Abstract

Water is the essential resource of all life. Humans, wildlife and entire ecosystems depend on a good quality of water resources. Therefore, the preservation and restoration of a good water quality in the world's environment is indispensable. However, humans are largely responsible for increasing contamination of this resource. It is generally accepted that this contamination needs to be reduced. Thus, the protection of surface waters and groundwater is regulated in various guidelines and directives like the EU Water Framework Directive (WFD). The required assessment of the status of the waterbodies includes knowledge about the various types of contamination. In this thesis, two different pollutants negatively affecting surface water quality are studied, namely residues of pharmaceutics and fecal contamination by bacteria.

Worldwide, the well-being of millions of people depends on pharmaceuticals to prevent and treat a variety of diseases. With greater accessibility and increased application frequency, more and more pharmaceutical residues are entering the water cycle, where they may cause adverse effects on aquatic wildlife. Fecal contamination of surface waters poses a specific threat to humans, for example, through contamination of rivers used for wild swimming. This type of contamination is usually assessed by the concentration of fecal indicator bacteria. In this thesis, fecal indicator bacteria were investigated with a special focus on antibiotic-resistant bacteria, as they are particularly risky for humans. Infections caused by antibiotic-resistant bacteria might be difficult to treat or not curable at all.

In this work, concentrations of pharmaceuticals and (antibiotic-resistant) fecal bacteria in surface waters of whole catchments were simulated to provide a basis for risk assessment. Concentrations were simulated using the geography-referenced regional exposure assessment tool for European rivers (GREAT-ER). Four research articles are included to (i) present the current publicly available version of the GREAT-ER model, (ii) conduct an extensive risk assessment of human-use pharmaceuticals in a cross-border catchment, (iii) apply the GREAT-ER model for the first time to simulate the fate of (antibiotic-resistant) Escherichia coli (*E. coli*) bacteria (as an indicator bacterium for fecal contamination) in surface waters in deterministic and (iv) stochastic simulations.

In the cross-border study area, investigation of pharmaceutical residues shows that safe ecological concentration limits are likely to be exceeded at least temporarily for diclofenac, carbamazepine, and 17α-ethinylestradiol, which are not regulated by the WFD. Likewise, the study highlights the importance to investigate (sub-)catchments across national boundaries. The application of the GREAT-ER model to predict (antibiotic-resistant) *E. coli* concentrations in river catchments demonstrates opportunities and limitations of the model with respect to its originally not intended application to bacteria. Model

results can serve as a basis to assess river catchments in terms of fecal contamination. These results suggest that swimming in waters influenced by wastewater treatment plant effluents is not advisable year-round and that the uptake of antibiotic-resistant bacteria cannot be ruled out when swimming in these waters. Under average conditions, measured concentrations are well represented by the model, while it reaches its limits under extreme conditions. Extending the model for a stochastic simulation routine using the Monte Carlo approach, allows for adequate predictions of the range of measured *E. coli* concentrations. With this approach, also key drivers of the spread of predicted concentrations could be identified.

Overall, the presented research highlights the strengths of predictive models in general and of GREAT-ER in particular for exposure assessment of contaminants in river basins and the advantage of the complementary approach of modeling in combination with monitoring.

Contents

Abbreviations

1D	one-dimensional
2D	two-dimensional
API	active pharmaceutical ingredient
AR	antibioc-resistent
ARB	antibioc-resistent bacteria
BMBF	Bundesministerium für Bildung und Forschung (Federal Ministry of Education and Research)
BWD	Bathing Water Directive
CAS	conventional activated sludge
CDF	cumulative distribution function
CEC	contaminants of emerging concern
COVID-19	Coronavirus disease 2019
CPE	carbapenamase-producing Enterobacteriaceae
CP-EC	carbapenemase-producing *E. coli*
CRED	criteria for reporting and evaluating ecotoxicity data
CSO	combined sewage overflow
DDD	defined daily dose
DNA	deoxyribonucleic acid
E. coli	Escherichia coli
E1	Estrone
E2	Estradiol
EARS-Net	European Antimicrobial Resistance Surveillance Network
EC	European Comission

ECDC	European Centre for Disease Prevention and Control
EE2	ethinylestradiol
EEA	European Economic Area
EFTA	European Free Trade Association
EQS	environmental quality standard
ERA	environmental risk assessments
ESBL-EC	extended spectrum beta-lactamase producing *E. coli*
EU	European Union
GE	Germany
GIS	geographic information system
HGT	horizontal gene transfer
HIV	human immunodeficiency virus
HRU	hydraulic response unit
HyReKa	Hygienisch-medizinische Relevanz und Kontrolle Antibiotika-resistenter Krankheitserreger in klinischen, landwirtschaftlichen und kommunalen Abwässern und deren Bedeutung in Rohwässern (Hygienic-medical relevance and control of antibiotic resistant pathogens in clinical, agricultural and municipal wastewaters and their significance in raw waters)
IQVIA	I (IMS Health), Q (Quintiles), and VIA (by way of)
IUSF	Institut für Umweltsystemforschung (Institute of Environmental Systems Research)
JRC	Joint Research Center
LOQ	limit of quantification
MAM10	mean annual 10-day minimum flow
MBR	membrane bioreactor
MEC	measured environmental concentration
MEDUWA	Medicines Unwanted in Waters
MRSA	methicillin resistant staphylococcus aureus
NL	The Netherlands

OECD	Organisation for Economic Co-operation and Development
PDF	probability distribution function
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
RQ	risk quotient
SFK	Stichting Farmaceutische Kengetallen (Foundation for Pharmaceutical Statistics)
SSD	species sensitivity distribution
STP	sewage treatment plant
USEPA	United States Environmental Protection Agency
VRE	vancomycin-resistant enterococci
WFD	Water Framework Directive
WHO	World Health Organization
WIS	Watershed Information System
WWTP	wastewater treatment plant

1. Introduction

1.1. Motivation

For years, the consumption of pharmaceuticals has been increasing worldwide. In 2019[1], 1.8 trillion defined daily doses (DDD)[2] of pharmaceuticals were consumed worldwide which corresponds to a 16% increase over five years. Approximately one-third of the doses are consumed in the countries of the top ten developed pharma markets, including several countries in the European Union (EU) (Kleinrock and Muñoz, 2020). Within the EU, more than 3 000 different active substances are authorized for medical use in human and veterinary medicine (European Commission, 2022).

The widespread use of pharmaceuticals resulted in the ubiquitous detection of pharmaceutical residues in the environment (Rivera-Utrilla et al., 2013). In an extensive review, aus der Beek et al. (2016) collected globally available monitoring data of pharmaceuticals in different environmental matrices and wastewater treatment plants (WWTPs). In the updated version, the detection of 771 different pharmaceuticals in samples from 75 countries covering all continents is reported (Dusi et al., 2019). Due to their broad distribution in the environment and their potential impact on human and ecosystem health, pharmaceuticals have been recognized as contaminants of emerging concern (CEC) (Daughton and Ternes, 1999; Halling-Sørensen et al., 1998; Patel et al., 2019; Sauvé and Desrosiers, 2014). Sauvé and Desrosiers (2014) define CEC as "naturally occurring, manufactured or man-made chemicals or materials which have now been discovered or are suspected to be present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being". Additionally, they suggest keeping the status "emerging" as long as potential consequences are unknown and a poor scientific database prevails. CECs are often characterized by low regulation, broad abundance in aquatic systems, resilience towards biological treatment, and persistence or slow degradation in the environment (Lee et al., 2021).

[1] Here, data from 2019 are presented since 2020 and 2021 consumption data are biased due to the COVID-19 pandemic (Muñoz, 2021).

[2] Defined daily dose (DDD) is a measure of pharmaceutical consumption introduced by the world health organization (WHO) for objectification and comparability of data in surveillance and research (WHO Collaborating Centre for Drug Statistics Methodology, 2020).

Among pharmaceuticals in the environment, antibiotics are of special concern, as the widespread use of antibiotics has been accompanied by a growing abundance of resistant strains (Davies and Davies, 2010). The World Health Organization (WHO) declared the antimicrobial resistance as one of the most severe public health threats humanity is facing (WHO, 2021b). The Organisation for Economic Co-operation and Development (OECD) estimates 700 000 deaths annually due to infections with multi-resistant pathogens on global level. The average prevalence of antibiotic resistance in bacterial infections increased from 10% to 15% in OECD member states between 2005 and 2014 (OECD, 2016). The European Centre for Disease Prevention and Control (ECDC) runs a system to monitor prevalence of antibiotic-resistant (AR) bacteria in the European Union (EU) and in European Economic Area (EEA) countries: the European Antimicrobial Resistance Surveillance Network (EARS-Net) (European Centre for Disease Prevention and Control, 2020). Cassini et al. (2019) evaluated EARS-Net data and found that the disease burden of AR pathogens is comparable to the combined burden of influenza, tuberculosis and the human immunodeficiency virus (HIV) in 2015 within EU/EEA. In the same year, 6.44 deaths per 100 000 population were attributed to AR bacteria (Cassini et al., 2019). Investigating all potential bacteria of interest is virtually impossible. The same holds true for AR bacteria. More than 200 different bacterial species have been identified in the human intestine alone (Loftus et al., 2021). The focus of this thesis is on fecal bacteria and therefore related to the fecal contamination of surface waters. Thus, the following model organisms were selected: i) Escherichia coli (*E. coli*) which are colonizers of the intestine of warm-blooded animals (including humans), among the best-studied organisms, and a frequently used indicator for fecal contamination; ii) extended spectrum beta-lactamase producing *E. coli* (ESBL-EC), a resistant subtype of *E. coli*, suggested as indicator organism to measure levels of antibiotic resistance by the WHO (WHO, 2021a); and iii) carbapenemase-producing *E. coli* (CP-EC), another resistant subtype of *E. coli*. Infections caused by carbapenamase-producing *Enterobacteriaceae* (CPE) have been identified as threatening infectious disease due to the high mortality rates (Nnadozie and Odume, 2019).

In order to assess potential effects of surface water pollution by pharmaceuticals or fecal contamination, a risk assessment is vital. For the assessment of in-stream concentrations of pharmaceuticals, environmental risk assessments (ERA) are a useful approach. Such ERAs usually combine two components for one contaminant: Environmental concentrations and effect thresholds. Environmental concentrations can be derived by measurements (measured environmental concentration, MEC) or by model predictions (predicted environmental concentration, PEC). For the prediction of pharmaceutical concentrations in surface waters, multimedia fate models and in-stream water quality models can be

utilized (Wind, 2004). In-stream models differ from multimedia models such as the Mackay models (Mackay, 2001) by their spatial reference, i.e. geography-referenced (geo-referenced) models. Such models are implemented as simulation programs in geographic information systems (GIS) which allows for a visualization of predicted concentrations in form of geographic river maps (Wind, 2004). In these models, transport is explicitly simulated in a one-dimensional or two-dimensional environment. Such spatially explicit approaches are more data-intensive but allow for the determination of spatial and temporal variation (Keller, 2006). Geo-referenced in-stream water quality models, the so-called "catchment models" consider the hydrology of an entire river catchment. A catchment (or watershed) model includes a representation of the river network and the sub-basins as well as the properties of its entities within the drainage basin of a river.

A catchment model which has been used for the exposure assessment of chemicals for more than two decades is the geography-referenced exposure assessment tool for European rivers (GREAT-ER) (Boeije et al., 1997; Feijtel et al., 1997). The model has been used primarily to simulate cleaning agent ingredients (e.g. Schowanek and Webb, 2002; Schulze and Matthies, 2001; Verdonck et al., 1999; Wind et al., 2004), the antimicrobial and antifungal agent triclosan (e.g. Capdevielle et al., 2008; Price et al., 2010; Sabaliunas et al., 2003), and pharmaceuticals (e.g. Alder et al., 2010; Cunningham, 2008; Johnson et al., 2007; Kehrein et al., 2015; Schowanek et al., 2001). The GREAT-ER model was expanded by including diffuse emissions to simulate emissions and fate of zinc and copper for the Ruhr River basin (Hüffmeyer et al., 2009). Since its development, the model has been applied to a number of catchments in different European countries (Aldekoa et al., 2013; Alder et al., 2010; Cunningham, 2008; Holt et al., 2003; Schowanek and Webb, 2002), and several catchments outside of Europe (Archundia et al., 2018; Hanamoto et al., 2013; Hannah et al., 2009; Hao et al., 2015; Jackson, 2018; Zhang et al., 2015).

The fecal contamination of surface waters (indicated by *E. coli* concentrations) is usually not assessed on a catchment scale, not to speak of the assessment of AR bacteria, where only one study has ever been performed on the scale of an entire catchment . This can be partially explained by the fact that threshold values for fecal contamination so far only exist for vulnerable areas such as swimming sites or drinking water abstraction sides, and that no threshold values for AR bacteria exist at all (Serwecińska et al., 2021). Nevertheless, there is large public interest in the dispersal and transport of resistant bacteria because swimming does not only take place at designated swimming areas (Falgenhauer et al., 2021; Uijtewaal and Amador, 2021; Wuijts et al., 2020). Since monitoring of bacteria in surface waters is laborious, time-consuming and costly, catchment models are helpful in providing a fast and comprehensive overview of the possible contamination with fecal and AR bacteria in a catchment.

1.2. Aims of the thesis

Catchment models are a valuable tool for the exposure assessment of chemicals in whole river basins. In recent years the GREAT-ER model was consecutively further developed. However, GREAT-ER is still an expert tool. The first objective is therefore to present the current version of the GREAT-ER model (version 4.1) for simulating pharmaceuticals in the environment and to make it readily accessible for interested users from science and administration.

The second objective is to perform an extensive environmental exposure assessment for human-use pharmaceuticals in a German-Dutch cross-border catchment. Thereby the knowledge base for exposure assessment of pharmaceuticals in whole river catchments will be extended. The investigated pharmaceuticals differ in their level of investigation, consumption patterns (among each other and between countries), fate in the human body, WWTPs and the waterbodies, and expected environmental concentrations. Furthermore, the combination with a risk assessment provides the basis to initiate action by authorities, if necessary.

The third objective is to simulate the status of fecal contamination of waterbodies on a catchment scale with the GREAT-ER model. Fecal contamination will be examined by modeling *E. coli* and AR *E. coli* concentrations. The modeling approach shall enable for a prioritization of emission sources and also for investigation of the influence of different (simulation) parameters on the range of the predicted concentrations. Since this is the first time that the fate of *E. coli* and AR *E. coli* will be simulated using the GREAT-ER model, opportunities and limitations of the approach shall be evaluated. Eventually, the suitability of simulated (AR) *E. coli* concentrations to perform an exposure assessment for wild swimming shall be investigated.

1.3. Structure of the thesis

Chapter 2 illustrates the scientific background of this thesis. This includes an overview regarding the current situation of pharmaceuticals in the aquatic environment (Section 2.1), emphasizing the importance of pharmaceutical fate and transport research. Moreover, the relevance of investigating fecal contamination, especially by antibiotic-resistant bacteria, in the environment will be highlighted (Section 2.2). This includes information on emission sources, resistance development and transfer, and the role of the environment in these processes. Furthermore, the model organisms studied in this thesis are introduced. Lastly, an overview of different catchment models is provided and the GREAT-ER model

is described more thoroughly (Section 2.3). Relevant European regulations and policies are presented since the case studies are performed in Germany and the Netherlands. Four scientific articles have been published to fulfill the objectives, shortly described in Chapter 3. The articles themselves build Chapters 4–7. Within the scope of this thesis it was necessary to extend the existing GREAT-ER software by a number of technical features. These extensions are described in Chapter 8. Finally, the results found in this thesis are discussed followed by an outlook (Chapter 9).

2. Background

2.1. Pharmaceuticals in the aquatic environment

2.1.1. Sources of emission to the aquatic environment

Pharmaceuticals may enter the environment via different pathways. Li (2014) identified six major sources of pharmaceutical emission which can be categorized into point and diffuse sources. Point sources include hospital, industrial and domestic wastewater. Diffuse sources on the other hand include agricultural runoff, i.e. runoff from pastures and agricultural areas after manure application, urban runoff from landfill, and leakage from the sewer and wastewater treatment systems. This thesis is limited to the investigation of human-use pharmaceuticals emitted by point sources. To provide a more comprehensive picture however, the other emission pathways are presented in this Chapter as well.

Wastewater originating from households or hospitals is usually collected in sewer systems and treated in wastewater treatment plants (WWTPs) which then constitute point sources for the aquatic environment. Domestic and hospital wastewater often contain pharmaceutically active substances, because these are not completely metabolized after application (Santos et al., 2010; Sim et al., 2011). Many pharmaceuticals are only used to a minor extent in hospitals, but for some the fraction of hospital application can be up to 80% (Alder et al., 2006). WWTPs are usually not designed to remove pharmaceuticals from collected wastewater as they were built to remove biodegradable organic compounds, nutrients (nitrogen and phosphorus compounds), and microbial organisms. Daily discharges of pharmaceuticals into receiving waters can vary by several orders of magnitude. For example, emission rates of 0.4 mg cap^{-1} d^{-1} have been reported for the antihypertensive hydrochlorothiazide and the psychiatric drug carbamazepine, while much lower emission rates (< 0.01 mg cap^{-1} d^{-1}) have been reported for the anti-inflammatory acetylsalicylic acid (also known as aspirin), and the antibiotic doxycycline (Verlicchi et al., 2012). In general, in WWTP effluents and in surface waters, human-use pharmaceutical concentrations are usually in the concentration range of ng L^{-1} to mg L^{-1} (Kümmerer, 2009). Leachate of pharmaceuticals from domestic waste in landfills as well as emissions caused

by leakage of the sewer system are considered minor emission sources to surface waters (Li, 2014).

Similar to human-use pharmaceuticals, veterinary pharmaceuticals are incompletely metabolized and detected in animal feces and manure (Ghirardini et al., 2020). In aquaculture, residues are directly emitted into surface waters. This pathway appears to be especially important in the Asia-Pacific region where 92% of global aquaculture production and 93.8% of global antibiotic consumption in aquaculture takes place (Naylor et al., 2021; Schar et al., 2020). Environmental exposure of pharmaceuticals due to livestock application mostly occurs due to direct drop-off of feces by grazing livestock or application of manure on agricultural areas. Pharmaceutical loads that enter the fields via manure depend on the management practices, type of livestock, and manure storage management. In addition, the application of manure is often limited by legal constraints (Ghirardini et al., 2020). Pharmaceuticals on the fields can enter surface waters and groundwater via runoff, infiltration and erosion depending on the molecular properties and the compound-soil interaction (Kaczala and Blum, 2016).

2.1.2. Risks emerging from pharmaceutical exposure

Pharmaceutical concentrations in drinking water are usually well below therapeutic concentrations (Patel et al., 2019), so that acute risks emerging from human exposure towards pharmaceuticals in drinking water can be considered low as well. However, long-term effects from chronic exposure, mixture effects, and the influence of the recipient's age are largely unknown (Kümmerer, 2009; Patel et al., 2019).

The environmental impact of pharmaceuticals has been widely recognized and addressed (Brausch et al., 2012; Halling-Sørensen et al., 1998; Pereira et al., 2020, e.g.). Two of the most prominent examples of pharmaceutical toxicity in the environment relate to the effects of the anti-inflammatory diclofenac on vulture populations in Southeast Asia and the feminization of fish populations by the contraceptive pill hormone ethinylestradiol (EE2) (Sumpter, 2007).

Diclofenac has been widely used to treat sick livestock in Southeast Asia. Oriental white-backed vultures fed on the dead livestock and thereby consumed diclofenac which still remained in the carcasses. The ingested diclofenac caused renal failure and death in the vulture populations (Oaks et al., 2004). This exposure pathway ultimately led to a decline of the oriental white-backed vultures and other vulture populations of more than 99% in Southeast Asia (Kümmerer, 2010).

The contraceptive pill hormone EE2 has been prescribed since the 1960s (Gruhn and Kazer, 1989). Purdom et al. (1994), found that EE at a concentration of 0.1 ng L^{-1}

resulted in a functional response in rainbow trout. Exposure of fathead minnow to a concentration of 4.0 ng L^{-1} resulted 56 days posthatch in a female-to-male sex ratio of 84:5 (Länge et al., 2001).

Among pharmaceuticals, antibiotics are active ingredients of particular concern. Besides ecotoxicological effects, antibiotic residues can foster antimicrobial resistance in the environment. This topic is further addressed in Section 2.2.1.

2.1.3. Regulation and surveillance of pharmaceuticals in surface waters

Worldwide, there are different national and regional guidelines for the protection of surface waters. A collection is presented in UN-Water (2015). EU member states are committed to implement the Water Framework Directive (WFD) to maintain or restore good ecological and chemical status of surface and ground waters at European level (European Union, 2000). A good chemical status encompasses that concentrations of so-called priority substances do not exceed environmental quality standards (EQS) (European Union, 2008). The initial list comprised 33 substances and was extended by 12 substances in 2013 (European Union, 2013). The pharmaceuticals EE2, estradiol (E2), estrone (E1), and diclofenac were proposed for inclusion but were assigned to a watch list instead along with the macrolide antibiotics erythromycin, clarithromycin and azithromycin (European Union, 2015). The purpose of the watch list is to gain better insights of the risk emerging from environmental concentrations of the respective compounds. Therefore, EU member states have to run monitoring programs addressing the watch list compounds, with measurements at least once per year for up to four years.

Since the establishment of the watch list, it has been updated twice (Cortes et al., 2020; Loos et al., 2018). As the data basis was considered sufficient, diclofenac was removed from the list in 2018. The hormones EE2, E1 and E2 and the macrolide antibiotics were removed two years later. Instead, the antibiotics amoxicillin, ciprofloxacin, sulfamethoxazole, trimethoprim and the antifungals clotrimazole, fluconazole and miconazole were added (European Union, 2018, 2020). The data collected during this period are utilized in the review process of the priority substance list. On the draft list for potential priority substances, only compounds with a "high" or "very high" risk are included. In a modeling study, diclofenac and E2 were assigned an "intermediate" risk in the latest review in 2016. EE2 was not considered for inclusion due to an insufficient data basis (Carvalho et al., 2015). In conclusion, there are currently no legal boundaries set by the EU for residues of pharmaceuticals in surface waters. However, it has been recognized by authorities that some pharmaceuticals in the aquatic environment pose a potential risk.

2.2. Antibiotic-resistant bacteria in the aquatic environment

2.2.1. Antibiotic resistance - development and acquisition

Natural antibiotics and antibiotic resistance genes exist since billions of years (Wintersdorff et al., 2016). The first antibiotic discovered by humans was the antibiotic penicillin by Alexander Fleming in 1928. A bacterial penicillinase - enabling the bacteria to escape the mechanism of penicillin - was discovered in 1940, a few years before penicillin was widely prescribed (Davies and Davies, 2010). Shortly after the market introduction, penicillin resistance became a serious problem in the therapy leading to the exploration and discovery of new antibiotics. By today, resistances to nearly all antibiotics that have been developed have been detected (Ventola, 2015). Resistance of a single microorganism to multiple antibiotics was first observed in the late 1950s to early 1960s. In the worst case, none of the antibiotics used to treat a bacterial infectious disease is effective if such multiple resistances are present (Levy and Marshall, 2004).

The rate of antibiotic resistance development and its spread has increased drastically in the last decades (Wintersdorff et al., 2016). As a result, infections with bacteria are once again a threat of growing concern (Ventola, 2015). The abundance of resistant bacteria is specifically increasing in environments where selective pressure favors resistant bacteria; i.e. in environments where they are exposed to antibiotics (Aslam et al., 2018). Since the introduction of antibiotics, millions of tons of antibiotic molecules have been produced and applied in various sectors. Cheaper production and higher accessibility allow for an increased application (Davies and Davies, 2010). Inadequate prescription procedures and application as poultry and livestock growth promoter fuel this development (Aslam et al., 2018).

In addition to the potential for the development of new resistances through *de novo* mutations, there is the possibility of horizontal gene transfer (HGT) (Marston et al., 2016). HGT involves the bacterial exchange or uptake of genes and encompasses various mechanisms: Transmission of genetic material by bacteriophages, exchange of mobile genetic elements, i.e. plasmids, the uptake of naked deoxyribonucleic acid (DNA), and transposons, i.e. DNA segments which can alter their position in the genome (Levy and Marshall, 2004). Conjugation, i.e. transmission via plasmids, is the most common HGT mechanism (Davies and Davies, 2010). It facilitates genetic exchange between different strains, species and even genera. One of the most recent examples of this phenomenon concerns the $bla_{\text{CTX-M}}$ gene which is now ubiquitous in humans, animals, and the environment (Wintersdorff et al., 2016).

2.2.2. Important multi-resistant bacteria

Among gram-positive pathogens, methicillin resistant staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) pose huge challenges to human health and modern medicine. The situation with MRSA is especially alarming, as MRSA spread in different epidemiological settings and do not only target health care associated environments. In contrast, VRE is mostly limited to clinical settings. However, only a few drugs retain antibiotic activity towards VRE (Rossolini et al., 2014). Also the treatment of infections with gram-negative pathogens is a growing challenge, because they become resistant to an increasing number of antibiotics. Among them, *Enterobacteriaceae* (e.g. *Klebsiella pneumoniae*, *E. coli*), Pseudomonas aeruginosa, and *Acinetobacter* are responsible for most infections in health care settings (Ventola, 2015). Multidrug-resistant *Enterobacteriaceae* include the extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*. ESBL *E. coli* has been selected by the WHO as an indicator organism to monitor the magnitude and trends of the global antibiotic resistance problem (WHO, 2021b). These multi-resistant bacteria are already prevalent in the community worldwide. Bezabih et al. (2021) found in a literature review a cumulative global pooled prevalence of 16.5% of ESBL *E. coli* in the intestines of healthy humans. Furthermore, they report an upward trend between the years 2003–2005 and 2015–2018. Infections caused by ESBL *E. coli* lead to significantly more death cases than infections with other *E. coli* due to the delay in initiating therapy with an appropriate antibiotic (Melzer and Petersen, 2007). For the treatment of infections caused by ESBL-producing *Enterobacteriacea*, carbapenems are the antibiotics of choice. Carbapenemase-producing *Enterobacteriaceae* are additionally resistant towards carbapenem antibiotics and are thus particularly worrisome (Rodríguez-Baño et al., 2018).

2.2.3. Role of the environment

Antibiotics, antibiotic resistance genes and antibiotic-resistant bacteria are regularly introduced into the environment by human activities (Larsson, 2014). The entry of antibiotics into the environment has already been described in Section 2.1, and since antibiotic resistance genes and resistant bacteria are abundant in the fecal material of humans and animals, basically the same entry pathways prevail, i.e. via discharge of (treated) wastewater, aquaculture and agricultural inputs (Pruden et al., 2013). The latter include manure application and direct drop-off by grazing animals. Surface runoff can wash contaminants into adjacent surface waters (Hall et al., 2020; Jacobs et al., 2019). The ongoing release of antibiotics into the environment led to a constant selective pressure towards resistant strains (Davies and Davies, 2010). Once acquired, resistance is lost only slowly due to

minimal survival costs (Levy and Marshall, 2004). These factors (emission, selective pressure, slow loss of resistance) lead to an increasing abundance of resistant bacteria in the aquatic environment. In rivers and lakes up to 98% and up to 77% of total detected bacteria have been reported to be resistant against at least one antibiotic, respectively. Thus, the aquatic environment is an important reservoir for antibiotic resistance (Nnadozie and Odume, 2019). The previously mentioned $bla_{\text{CTX-M}}$ ESBL gene is one of the best-known examples of resistance genes which have been mobilized from environmental bacteria. Another example is the OXA-48 gene encoding for carbapenem resistance which has been of increasing importance (Wintersdorff et al., 2016).

The contamination of freshwaters with resistant bacteria poses direct and indirect threats for humans. Swimming in contaminated waters can lead to an uptake of AR bacteria (Mughini-Gras et al., 2019). Irrigation of plants with these waters followed by a consumption of the plants as well as consumption of contaminated water can also lead to an uptake of AR bacteria (Finley et al., 2013). While most of environmental bacteria are commensal (Abia et al., 2016) some of the resistant bacteria could be pathogenic (Schijven et al., 2015a). On the other hand, the uptake of resistant bacteria (commensal or pathogenic) can lead to HGT with bacteria already present in the intestines which could also be pathogenic themselves (Finley et al., 2013).

2.2.4. Threshold values

In order to protect people from the ingestion of pathogenic bacteria via water, there are various protective measures in the EU. However, none of them was developed for protection against resistant bacteria, but generally for protection against bacterial infection. For example, the EU-Drinking Water Directive sets a limit value of 0 CFU L^{-1} (European Union, 1998). The EU Bathing Water Directive defines limit concentrations for indicator organisms that indicate the degree of fecal contamination (European Union, 2006). For a "good quality", concentrations of intestinal enterococci and *E. coli* must be below 4 000 CFU L^{-1} and 10 000 CFU L^{-1}, based on a 90-percentile assessment. According to Exner et al. (2018), these limits are sufficient to exclude health risk from multi-resistant pathogens. Furthermore, there is currently no obligation to monitor surface waters for fecal contamination or antibiotic resistance in the EU, Germany, or the Netherlands. Nevertheless, the BMBF[1]-funded HyReKa[2] project recommends additional monitoring

[1]Bundesministerium für Bildung und Forschung; english: Federal Ministry of Education and Research

[2]Hygienisch-medizinische Relevanz und Kontrolle Antibiotika-resistenter Krankheitserreger in klinischen, landwirtschaftlichen und kommunalen Abwässern und deren Bedeutung in Rohwässern; english: Hygienic-medical relevance and control of antibiotic-resistant pathogens in clinical, agricultural and municipal wastewaters and their significance in raw waters

of antibiotics, indicator organisms and certain resistance genes in addition to the Water Framework Directive (HyReKa, 2020).

2.3. Chemical and bacteria fate modeling in catchments

2.3.1. Catchment models

Some catchment models are systems for simulating contaminants, others are incorporated into water quality system models, e.g. the Hydrological Simulation Program Fortran (HSPF) in the Watershed Modeling System (WMS) (AQUAVEO, 2022). There also are models which can be utilized both ways, e.g. the Soil and Water Assessment Tool (SWAT) which also is a sub-module in the Automated Geospatial Watershed Assessment Tool (AGWA) (U.S. Environmental Protection Agency, 2022a).

Catchment models can differ in their spatial and temporal resolution, in their complexity and in the representation of the catchment. A comparison of selected models is provided in Table 2.1. Complexity encompasses the level of detail and the number of processes incorporated in the derivation and representation of the watershed. Physically-based watershed models are rather complex. They derive hydrological processes and properties on the basis of associated physics (Daniel et al., 2011). Examples are HSPF and SWAT. In contrast, empirical models are regression based, e.g. FLO1K (Barbarossa et al., 2018) which is implemented in the ePIE (exposure to Pharmaceuticals in the Environment) model (Oldenkamp et al., 2018), and are thus in tendency less complex as compared to physically-based models. Model complexity also includes the spatio-temporal resolution. Temporally explicit models simulate catchment hydrology in time steps ranging from a few minutes, e.g. HSPF, to some days, e.g. SENEQUE/Riverstrahler (see Table 2.1). In LF2000-WQX (Low Flows 2000-Water Quality modelling eXtension) and GREAT-ER (geography-referenced regional exposure assessment tool for European rivers), temporal variability can be represented by describing parameters such as the flow rate via probability distribution functions, and simulations are performed as stochastic Monte Carlo simulations (Boeije et al., 1997; Keller and Young, 2004). Considering the spatial scale, models can be subdivided into lumped, semi-distributed and distributed models. In a lumped approach, whole catchments are modeled as an entity, and properties are averaged over this unit. Semi-distributed and distributed models account for spatial heterogeneity. Semi-distributed models sub-divide the catchment in smaller sub-catchments, whereas the spatial resolution of distributed models is usually defined by the modeler (Daniel et al.,

2011). In all these models, river channels are represented or aggregated in form of homogeneously mixed, one-dimensional segments with a property vector that includes the hydrological parameters. In HSPF and SWAT, the water segment is part of a so-called Hydraulic response unit (HRU). An HRU is an aggregation of the sub-basin of a river and can additionally contain data about land-use, soil properties, ponds, groundwater or reservoirs.

Temporally explicit models such as HSPF and SWAT have intentionally been developed to simulate the fate of nutrients and pesticides (Arnold et al., 1998; Donigian Jr et al., 1994; Srinivasan et al., 1998). However, they have been applied for the simulation of pharmaceuticals (Iavorivska et al., 2020; Zhao and Lung, 2017), and the SWAT model also for the simulation of *E. coli* (Kim et al., 2010). These models are primarily applied to the evaluation of contaminants from diffuse sources such as manure or slurry. Steady-state models such as ePIE, LF2000-WQX and GREAT-ER have been developed to simulate the fate of down-the-drain chemicals, e.g. cleaning agent ingredients or human-use pharmaceuticals (Boeije et al., 1997; Oldenkamp et al., 2018; Price et al., 2010).

Table 2.1: Selected watershed models and watershed model systems (WMS). Adapted and extended from Daniel et al. (2011).

	HSPF	SWAT	SENEQUE/ Riverstrahler	LF2000-WQX	ePIE/FLO1K	GREAT-ER
Complexity	High	High	Medium to high	Medium	Low	Medium
Spatial scale, resolution	Semi-distributed (depends on HRU)	Semi-distributed (depends on HRU)	Distributed, 1 km	Distributed, river reaches visible at 1:50,000	Distributed, ∼1 km	Distributed, < 2 km
Temporal scale, resolution	Continuous, minutely to daily steps	Continuous, daily steps	Continuous, 10 day steps	Annual average flows (mean, low)	Annual/ monthly average flows (mean, low)	Annual average flows (mean, low)
Watershed representation	HRU, stream channels; 1-D channels	1-D main channel, HRU, ponds, groundwater	1-D channel segments, stream order	1-D channel segments	1-D channel segments	1-D channel segment, (wash-off areas)
Deterministic, stochastic	Deterministic, stochastic	Deterministic	Deterministic	Deterministic, stochastic	Deterministic	Deterministic, stochastic
Physics based, empirical	Physics-based	Physics-based	Physics-based, empirical	Physics-based, empirical	Empirical	Physics-based, empirical
Simulated compounds and organisms [a]	e.g. organic N/P, N/P, Pesticides	e.g. N, P, pesticides, C	e.g. N, P, plancton	Down-the-drain chemicals	Pharmaceuticals only	Down-the-drain chemicals, copper, zinc
Simulation of pharmaceuticals	Yes	Yes	No	Yes	Yes	Yes
Simulation of bacteria	Yes	Yes	Yes	No	No	No
WMS	No	Yes	Yes	Yes	Yes	Yes
References [b]	[1–4]	[5–8]	[9–12]	[13–14]	[15–16]	[17–20]

[a] N = nitrogen, P= phosphate, C = Carbon

[b] [1] Donigian Jr et al. (1994), [2] U.S. Environmental Protection Agency (2022b), [3] Zhao and Lung (2017), [4] Chin et al. (2009), [5] Arnold et al. (1998), [6] Srinivasan et al. (1998), [7] Kim et al. (2010), [8] Iavorivska et al. (2020), [9] Billen et al. (1994), [10] Garcia-Armisen et al. (2006), [11] Bultot and Dupriez (1976a), [12] Bultot and Dupriez (1976b), [13] Keller and Young (2004), [14] Johnson et al. (2007), [15] Barbarossa et al. (2018), [16] Oldenkamp et al. (2018), [17] Boeije et al. (1997), [18] Feijtel et al. (1998), [19] Hüffmeyer et al. (2009), [20] Kehrein et al. (2015)

2.3.2. GREAT-ER

Among the models presented, the GREAT-ER model software was selected to simulate the fate of pharmaceuticals and (resistant) *E. coli*. GREAT-ER was originally developed to predict and assess the fate of down-the-drain chemicals in whole watersheds (Boeije et al., 1997; Feijtel et al., 1997). The software includes a simulation routine, tools to analyze, evaluate and assess predicted concentrations as well as a scenario creator to calculate management scenarios (Kehrein et al., 2015). The model is based on two main assumptions: Steady state, and mass balance (Feijtel et al., 1997). Steady state means that processes remain constant over time, e.g. the assumption of constant emissions in the model. Mass balance means that no mass is lost within the model boundaries, i.e. the following equation is fulfilled for each segment of the river network:

$$0 = m_{in} - m_{out} - m_{dis} \tag{2.1}$$

where m_{in} is the mass which enters a segment, m_{out} is the mass which is forwarded to adjacent segments and m_{dis} is the mass which is dissipated in that segment, e.g. due to degradation. Internally, the model considers chemical masses and water volumes as rates, i.e. flows of mass and volume per time. Concentrations are calculated by dividing the mass flow (mass per time) with the water flow rate (water volume per time). The model is organized in three basic units: the hydrological model, the emission model and the chemical fate model. The hydrological model was introduced in Section 2.3.1 and is summarized in Table 2.1. The emission model includes chemical emissions by point sources (e.g. WWTPs, direct industrial dischargers) or by diffuse entries (e.g. wash-off from adjacent areas). In the fate model, a chemical compound can be subject to advective transport and different loss processes, e.g. sedimentation, volatilization, photolysis or hydrolysis, that depend on inherent compound properties, e.g. light adsorption characteristics, and hydraulic properties, e.g. depth and velocity (Feijtel et al., 1997; Hüffmeyer et al., 2009; Kehrein et al., 2015). Figure 2.1 illustrates processes considered in the GREAT-ER model.

There is a long history of development and application of the GREAT-ER model in the Institute of Environmental Systems Research (IUSF) at Osnabrück University (IUSF, 2022). The model has been practically applied to assess the chemical status of catchments, e.g. in Bavaria or North Rhine-Westphalia (Klasmeier and Berlekamp, 2017; Klasmeier et al., 2018). While the model concept is still the same, many improvements have been made to the software that affected model performance and data management. Additional sub-models have been implemented to simulate the fate of a variety of chemicals, e.g. metals, X-ray contrast agents or nutrients. Furthermore, tools for the analysis and evaluation of predicted concentrations have been implemented and extended, e.g. monitoring

comparison tools, concentration profiles or cumulative distribution functions. A detailed description of GREAT-ER history through 2015 (version 4) is provided in Kehrein et al. (2015). A collection of GREAT-ER model equations is presented in the PhD thesis by Lämmchen (2021a).

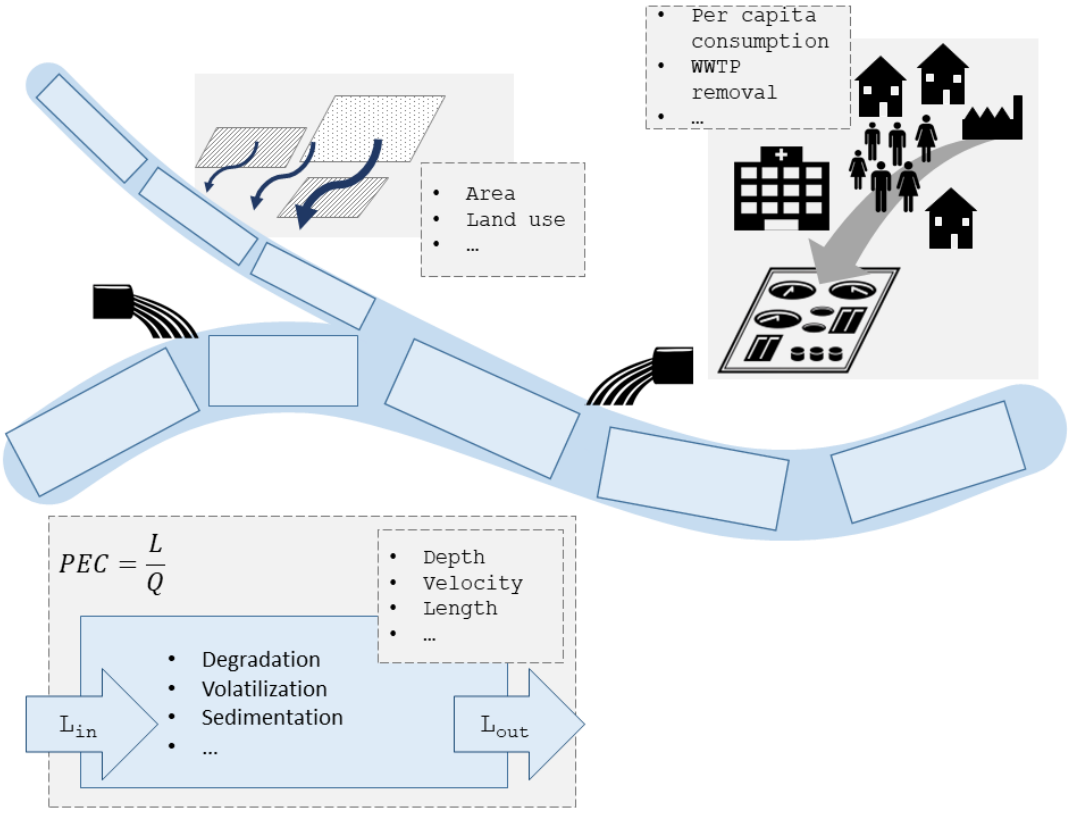

Figure 2.1: Conceptual representation of the GREAT-ER model. PEC: Predicted environmental concentration [mass per volume], L: mass flow [mass per time], Q: flow rate [volume per time].

2.4. MEDUWA project

The research described in this thesis was performed in the EU-INTERREG project MEDUWA (Medicines Unwanted in Water) Vecht. The overall objective of the project was to reduce the emission of pharmaceuticals into the environment. Solutions were to be developed along the entire lifecycle: From production of the pharmaceuticals to their emission into the environment. The Dutch-German cooperation project was carried out in the study area of the Vecht River, which has its source in Germany and flows into the Zwarte Water in the Netherlands. The project also aimed to determine the status quo of pharmaceutical pollution and the associated risk to humans and the environment in the Vecht catchment. Among the investigated pharmaceuticals, antibiotics play a special

role as their administration can help fighting diseases but also promote the development of resistant pathogens (Section 2.2.3). Therefore, the study of AR bacteria formed an essential part of the project.

Geo-referenced modeling played a vital role in the MEDUWA project as it allows for a spatially resolved representation of the pollution in a catchment. This information can be used for a risk assessment by comparing simulated concentrations with risk thresholds such as PNEC (predicted no-effect concentration) values, or with regulatory targets (e.g., environmental quality standards, EQS). Furthermore, the mitigation effects of the solutions proposed in the MEDUWA project for the reduction of pharmaceuticals and (AR) *E. coli* into the environment were simulated.

Results presented in this thesis were integrated into an online database platform: the Watershed Information System (WIS), developed by the GIS company Geoplex. In the WIS, project results are partially included, pooled and presented to the public in the form of interactive maps. This also includes the presentation of predicted pharmaceutical and resistant *E. coli* concentrations (Chapters 5–6). Additionally, potential effects of interventions developed in MEDUWA are displayed in the WIS. For this purpose, GREAT-ER was utilized to create action scenarios which simulate the effect of implemented interventions on predicted pharmaceutical concentrations. Thus, the WIS is both, a tool for science communication to the interested public and a tool to market mitigation strategies and products.

3. Summary of research articles

Article 1: Geo-referenced simulation of pharmaceuticals in whole watersheds: application of GREAT-ER 4.1 in Germany

Since the development of GREAT-ER (Feijtel et al., 1997), the GREAT-ER working group at the Institute for Environmental Systems Research (IUSF) has extended the model and adopted it to the needs of scientific and regulatory users (e.g. Klasmeier and Berlekamp, 2017; Klasmeier et al., 2018). This includes the development of new simulation routines as well as improvements of data storage and data processing. In this Chapter, the current state of the GREAT-ER model is demonstrated by simulating pharmaceuticals in different German catchments. The last published update of the GREAT-ER model was in 2015 with the development of GREAT-ER 4.0 (Kehrein et al., 2015). The changes made since then justify a new release and promotion of the current state of GREAT-ER in a version 4.1. In recent years, the model has been applied in cooperation with different authorities but was also requested from external researchers. Therefore, the aims of the study presented in this Chapter were to present newly adopted and improved features of GREAT-ER 4.1 as well as the features of a basic version of GREAT-ER which is more readily accessible for inexperienced users. The new simulation routines include an update of the hospital model for modeling pharmaceuticals, an additional flow situation (median discharge, Q50) as well as the development of new and improvement of existing analysis tools. To make the software more readily accessible, a basic version of the GREAT-ER model was built, accompanied by a detailed description of the pre-processing of data to set up databases for simulations with the GREAT-ER model.

My contribution to this work includes the adaption of the GREAT-ER software to the needs of the case studies. This includes the set-up of the basic GREAT-ER version as well as the build of GREAT-ER 4.1. Conceptual adaptions of the GREAT-ER model were performed by the entire GREAT-ER working group of the IUSF. Preparation and execution of case studies, the pre-processing guide as well as the writing of the original draft were carried out by V. Lämmchen who was in charge of this study. My contribution to reviewing and editing the manuscript was proportional to the co-authors.

Article 2: Ecological risk assessment of pharmaceuticals in the transboundary Vecht River (Germany/Netherlands)

In this Chapter, the GREAT-ER model is applied to predict spatially explicit concentrations of eight human-use pharmaceuticals in the catchment of the transboundary Dutch-German Vecht River with the aim to provide an environmental risk assessment for the selected pharmaceuticals. The hydrology of the case study catchment is highly influenced by anthropogenic activities, i.e. water is pumped through the catchment through a network of canals, especially in summer to keep water levels constant and prevent tributaries from falling dry. To conduct risk assessment in such a challenging catchment, pharmaceuticals were simulated in two distinct scenarios representing different hydrologic (average flow and low flow) and seasonal (in terms of sunlight intensity) conditions. Selected APIs represent a broad range of application volumes, consumption patterns in Germany and the Netherlands, therapeutic classes and chemical properties. Predicted concentrations are evaluated against measured values in WWTP influents and effluents as well as at selected river sites across the catchment. Eventually, the risk assessment is conducted by comparing predicted environmental concentrations (PECs) with predicted no-effect concentrations (PNECs).

The hydrologic model was set up in a previous work by Lämmchen et al. (2021b). My contribution to this work was in the adaption of the GREAT-ER software to efficiently process and evaluate scenarios in the transboundary catchment. Moreover, data acquisition and processing for model parameterization as well as scenario set-ups were performed collaboratively with V. Lämmchen accompanied by advisory exchange with J. Klasmeier. Calculation of seasonal surface photolysis rates were performed by J. Klasmeier. Furthermore, I was in charge of the model evaluation, i.e. comparing predicted WWTP loads and in-stream concentrations against measured values. The latter were obtained as part of a one year monitoring campaign in the Vecht catchment and provided by co-authors from WETSUS, European Centre of Excellence for Sustainable Water Technology. The monitoring campaign was developed in close cooperation of the working groups of the IUSF and WETSUS under the lead of E. van Heijnsbergen. The comparison of PECs and PNECs was performed jointly by the first authors. PNECs were derived by D. Duarte, R. Oldenkamp and A. Ragas. My contribution to the writing of the original manuscript and the reviewing and editing of the manuscript was proportional the first authors and all co-authors, respectively.

Article 3: (Antibiotic-resistant) *E. coli* in the Dutch-German Vecht catchment - Monitoring and modeling

The aim of this article is to provide a method to predict spatially explicit in-stream concentrations of *E. coli* and antibiotic-resistant (AR) *E. coli*, namely ESBL-producing *E. coli* and carbapenemase-producing *E. coli*, at the catchment level. This allows to provide an overview of the spatial distribution of (AR) *E. coli* concentrations in the catchment, and to identify important emission sources and hotspots. Such predicted concentrations can be utilized for a preliminary exposure assessment, e.g. for swimming, or the uptake of AR *E. coli* when swimming at hotspots. To achieve this objective, the GREAT-ER model was adapted to simulate (AR) *E. coli*. The selected study area is the same as in the second article, namely the catchment of the Vecht River. Thus, the same hydrological model (Lämmchen et al., 2021b) presented in Article 2 was adopted for this study. Analogous to the simulation of pharmaceuticals in the Vecht catchment, two scenarios are defined, one describing an average flow situation and one representing a dry summer. The parameterization of the *E. coli* model is based on monitoring and literature data. Emissions are estimated on the basis of data from the monitoring campaign and fate in the river network is estimated by parameters and models from case studies of fecal coliform bacteria and *E. coli* in the catchments of the rivers Seine and the Scheldt (Ouattara et al., 2011; Servais et al., 2007). The final model is evaluated by comparing predicted concentrations with measured concentrations from monitoring sites distributed over the catchment. An exposure assessment for fecal contamination is conducted based on the guidelines of the EU Bathing Water Directive (European Union, 2006). For AR *E. coli* maximum swallowed loads for a swimming event are calculated.

This article combines monitoring and modeling of (AR) *E. coli*. The monitoring campaign, which also included the pharmaceutical monitoring of the second article, was carried out by WETSUS, European Centre of Excellence for Sustainable Water Technology, under the direction of E. van Heijnsbergen, as described earlier. This included sampling, sample analysis, and data preparation as well as the generation of descriptive statistics. The statistical analysis of the bacteria samples in the WWTPs was performed by me under the supervision of H. Schmitt. Furthermore, I was responsible for the extension of the GREAT-ER model to simulate (AR) *E. coli*. This includes the conceptual setup of the model, the implementation, the parameterization and the evaluation of the model. This was carried out under the supervision of J. Klasmeier with regular exchange with E. van Heijnsbergen, L. Hernández-Leal and H. Schmitt. The manuscript was written by E. van Heijnsbergen and me and reviewed and edited by all co-authors.

Article 4: Monte Carlo Simulations of *E. coli* in a Sub-catchment of the Vecht River

In the third research article it is demonstrated under which preconditions *E. coli* and AR *E. coli* can be simulated in the Vecht catchment utilizing the GREAT-ER model. Due to the particular flow situation in the study area, it was initially only possible to represent river flow rates by long term averages in two scenarios. Therefore, microbial concentrations were simply modeled as average concentrations for the two flow situations. However, in-stream measurements of *E. coli* showed at some sampling sites a range of concentrations over far more than one order of magnitude. The goal of this research article is therefore to parameterize the newly introduced *E. coli* sub-model in GREAT-ER for Monte Carlo simulations. Thus, predicted microbial concentrations can be expressed as probability distributions, representing concentrations over the course of a year. This allows for the inclusion of both, parameter uncertainty and parameter variability. A sub-catchment of the Vecht River was selected as the study area. Here, a stochastic parameterization is feasible. By means of stochastic simulations the influence of the distribution of individual parameters on the width of predicted concentrations can be evaluated. Furthermore, by representing predicted concentrations over the course of the year, a percentile assessment of predicted *E. coli* concentrations based on the EU Bathing Water Directive (European Union, 2006) can be performed.

I was the major contributor in designing the methodology, conducting the study, evaluating the results, creating the simulation software, and writing the manuscript. The parameterization of the hydrological model based on the work of Lämmchen et al. (2021b) for the modeling of the sub-catchment under exchange with V. Lämmchen and J. Berlekamp. The *E. coli* sub-model was already implemented in GREAT-ER for the previous article. For the stochastic simulation, I adapted the GREAT-ER software to the needs of the study. The monitoring data for parameterization and evaluation of the model were taken from the previous article. Revision of the manuscript was conducted jointly by all co-authors.

Original publication: Environmental Science and Pollution Research International
28(17): 21926–21935 (2021)
available at http://dx.doi.org/10.1007/s11356-020-12189-7

Geo-referenced simulation of pharmaceuticals in whole watersheds: application of GREAT-ER 4.1 in Germany

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Abstract

The geography-referenced regional exposure assessment tool for European rivers (GREAT-ER) is designed to support river basin management or the implementation process within the EU Water Framework Directive by predicting spatially resolved exposure concentrations in whole watersheds. The usefulness of the complimentary application of targeted monitoring and GREAT-ER simulations is demonstrated with case studies for three pharmaceuticals in selected German watersheds. Comparison with monitoring data corroborates the capability of the probabilistic model approach to predict the expected range of spatial surface water concentrations. Explicit consideration of local pharmaceutical emissions from hospitals or private doctor's offices (e.g. for X-ray contrast agents) can improve predictions on the local scale without compromising regional exposure assessment. Pharmaceuticals exhibiting low concentrations hardly detectable with established analytical methods (e.g. EE2) can be evaluated with model simulations. Management scenarios allow for *a priori* assessment of risk reduction measures. In combination with targeted monitoring approaches, the GREAT-ER model can serve as valuable support tool for exposure and risk assessment of pharmaceuticals in whole watersheds.

4.1. Introduction

A major problem for humankind is access to clean and readily available drinking water. Therefore, protection of groundwater and surface water against unwanted and potentially harmful chemical contaminants is important. The European Water Framework Directive (WFD) constitutes a legal framework that imposes the protection of common water resources on European states (European Union, 2000). The call of the directive among other things is the good chemical status of European surface waters. To achieve this goal, exposure and risk assessment of micropollutants, including pharmaceuticals, followed by development and implementation of reduction measures for critical compounds is necessary. Currently, the WFD lists 45 priority substances in Annex X of the directive and sets environmental quality standards for each of these substances. A prerequisite for the definition and implementation of mitigation measures is knowledge of the exposure concentrations of chemicals in the aqueous environment. This has led to large monitoring efforts for so-called emerging contaminants such as pharmaceuticals. To focus these efforts on potentially harmful substances, a watch list was established in 2015 whose purpose is to enforce collection of concentration data for those emerging pollutants for which available monitoring data are considered insufficient. The first watch list included diclofenac, three hormones (estrone (E1), 17β-estradiol (E2), and ethinylestradiol (EE2)), and three macrolide antibiotics (erythromycin, clarithromycin, azithromycin). The list is regularly reviewed in order to respond to new information and to avoid monitoring of substances for longer than necessary. In a recent review conducted by the Joint Research Centre (JRC) of the EU, it was concluded that diclofenac could be removed and the updated list should instead include the two antibiotics amoxicillin and ciprofloxacin among thirteen other substances (Loos et al., 2018).

In the last years, numerous papers have been published demonstrating the ubiquitous presence of pharmaceutically active substances in surface waters all over the world (e.g. Chiffre et al., 2016; Ivesic et al., 2017; Nebot et al., 2015). The monitoring data show a large variability of micropollutants' surface water concentrations in time and space. Consequently, each data point should always be interpreted in relation to environmental conditions during sampling, e.g. values of key parameters such as river flow. However, it is obvious that permanent basin-wide monitoring of thousands of possible contaminants is virtually impossible. Moreover, even if selection of sampling sites has been done considering local circumstances, spatial variability of the monitoring results can often not be satisfyingly explained. At this point, geo-referenced simulation models can be of great help for exposure and risk assessment such as the GREAT-ER model (Kehrein et al., 2015). Other prominent examples are substance flow models set up for Switzerland

(Kuroda et al., 2016; Ort et al., 2009) and the Netherlands (Coppens et al., 2015) or the LF2000-WQX water quality model (Price et al., 2010).

The well-established model GREAT-ER (geography-referenced regional exposure assessment tool for European rivers) predicts spatially resolved exposure concentrations for down-the-drain chemicals (Aldekoa et al., 2013; Alder et al., 2010; Feijtel et al., 1998; Kehrein et al., 2015; Koormann et al., 2006). Simulation results can be used to easily identify river sites where elevated concentrations, e.g. above a defined target value (PNEC or EQS), are expected. This information can support targeted selection of sampling sites and compliment the interpretation of monitoring data in terms of plausibility. Additionally, simulations of management scenarios for selected reduction measures and *a priori* evaluation of their effectiveness can be very helpful for water managers.

The objective of this paper is to illustrate the capabilities and limitations of GREAT-ER 4.1 using meaningful case studies for selected pharmaceuticals in three different German catchments. In particular, we demonstrate (1) the usefulness of the probabilistic model approach to consider natural variability of river flow that is reflected by the temporal variability of measured concentrations at selected sites, (2) the explicit consideration of hospital wastewater emissions important for pharmaceuticals predominantly emitted at the location of treatment, (3) basin-wide exposure assessment for substances with low PEC and EQS values, and (4) the informative value of management scenario simulations.

4.2. Material and methods

4.2.1. The GREAT-ER 4.1 model software

How the model works

The GREAT-ER model calculates spatially explicit steady-state concentrations of down-the-drain chemicals in surface waters of entire catchment areas considering point and non-point emissions from different sources assuming more or less constant emissions over time (Hüffmeyer et al., 2009; Kehrein et al., 2015). In general, wastewater from households, hospitals, and industry as well as runoff from agricultural areas can be taken into account as emission sources. Household emissions are treated according to the place of residence using an average per capita consumption value. In GREAT-ER 4.1, a hospital sub-model to investigate the local effect of hospital wastewater on the concentrations of selected medicinal agents has been adopted. The number of total patients (or beds) in hospitals has been suggested as appropriate proxy for respective emissions from a single hospital

(Kuroda et al., 2016). Therefore, GREAT-ER 4.1 requires a per patient consumption value in this case.

The model uses mass balance equations that track the chemicals along the emission pathways into surface water including removal in wastewater treatment plants (WWTPs). Sedimentation, volatilization, and degradation by photolysis, hydrolysis, or biological processes are considered as pseudo first-order in-stream loss processes. Mass conservation applies to each segment, so that the mass flow at the beginning corresponds to the mass flow at the end, unless it has been changed by diffuse emissions or loss processes. In the model, the river network is represented as a hydrological geometric network which is subdivided into segments (edges) of maximum length of 2 000 m. Nodes are set at all confluences, point emission sites, and other points of interest (e.g. gauges, monitoring sites, weirs). Emission loads from point sources (mainly WWTPs) are estimated by a series of submodules. The loads are discharged into the receiving river at the respective node and are then transported further downstream in the model. Loads are expressed in terms of mass per unit time and are considered constant over time in order to obey to the steady-state assumption. The model requires a number of substance-specific input parameters as well as environmental attributes. This encompasses physicochemical data, consumption, and use patterns as well as removal efficiencies during sewage treatment. The latter is modeled as simple percentage removal whose efficiency depends on the specific treatment category (lagoon, constructed wetland, bio filter, or activated sludge). Each river segment possesses a vector of attributes, e.g. flow velocity and river flow, which is used for the calculation of required intermediate parameters such as travel time. Depending on the available information, the user can choose between different complexity modes for the different submodules. A detailed description of the model equations is given in the appendix of Kehrein et al. (2015).

Natural variability of environmental parameters, uncertainty of substance parameters, and temporal fluctuation of consumption patterns can be considered by a probabilistic Monte Carlo approach. As opposed to deterministic model runs, corresponding parameters are not fixed, but defined as probability distributions of random variables. The distributions represent the expected frequency with which a parameter will take a single value. Probabilistic model runs are performed iteratively with parameter value vectors chosen from the probability distributions. The model calculates concentration distributions for each river segment mapping the expected range of the temporal variability for the selected parameter combinations. The output can be used to calculate any percentile of the respective concentration distribution. Results are primarily presented as color-coded maps or concentration profiles along a selected river course (see Figures 4.2 and 4.4 in the case study section). In addition, a number of options for in-depth analyses of the results

are implemented. Another key feature of the GREAT-ER model is the scenario builder. It enables the user to evaluate the effect of defined changes in boundary conditions on the simulated concentrations. Potential scenarios include changes in consumption, technical retrofitting of sewage treatment plants (tertiary/quaternary treatment), or re-routing of wastewater.

How to prepare a GREAT-ER database

The GREAT-ER model core is delivered as Add-In for the commercial software ArcGIS Desktop®. The GREAT-ER philosophy follows the idea of river basin management as laid out in the EU Water Framework Directive. This means that model simulations are performed within whole catchments including all watercourses with perennial flow. All required data for the simulations must be stored in a catchment-specific database. The databases need to have a standardized structure, which is assigned during the so-called pre-processing. Here, raw data are processed to form the topological river network, to connect point sources (WWTP, industry, and hospitals), and to assign other data (gauges, monitoring sites) to the respective river segments.

Over the years, GREAT-ER has become increasingly complex due to new simulation and analyses features to fulfil the needs of different users such as scientists, authorities, (environmental agencies) and industry, and the demand for the tool has continuously increased. However, one of the major obstacles for widespread use of the model was the laborious preparation of the required data set for the catchment under investigation. Preparation of an executable database for a selected river basin demands a number of pre-processing steps, which has so far impeded broad application of the model by different users. This problem has been partly overcome since the freely available model version now comes along with a semi-automated data processing routine for catchment preparation, several tutorials, and an exemplary dataset of a hypothetical catchment with which users can set up a GREAT-ER database and familiarize themselves with its practical use. This forms a sufficient knowledge base for interested users to generate their own catchment database and proceed with the full version GREAT-ER 4.1.

A prerequisite for GREAT-ER simulations is assignment of realistic flow rates for average conditions (MQ), dry weather (MNQ), and the 50^{th} percentile (Q50) to each river segment. There are numerous hydrological models (e.g. SWAT or NASIM) that can be used to estimate these data independently and import them into the GREAT-ER database. The GREAT-ER pre-processing provides an alternative semiautomated procedure to estimate river flow for each segment from spatially resolved runoff data for the whole catchment. Regardless how the MQ and MNQ values for each segment were estimated, they are

calibrated against available gauging data before use. Substance-specific parameters have to be entered manually into the respective fields of the database. Selected attributes in the database (e.g. number of people connected to a treatment plant) can be edited to keep it up-to-date.

4.2.2. Case Study simulations

For the application of the model, three different pharmaceutical compounds in three German river basins of different size (see Figure 4.1) have been simulated. The specific characteristics make them suitable to demonstrate some of the main benefits of the new model version for exposure (and risk) assessment. The selected substances were the antibiotic clarithromycin, the X-ray contrast agent iopamidol, and the natural hormone ethinylestradiol (EE2). All simulations were performed applying the implemented Monte Carlo simulation routine with 10 000 model realizations. All substance properties used for the model simulations are given in Table A.1. The location of the three catchments is shown in Figure 4.1; basin characteristics are summarized in Table A.2.

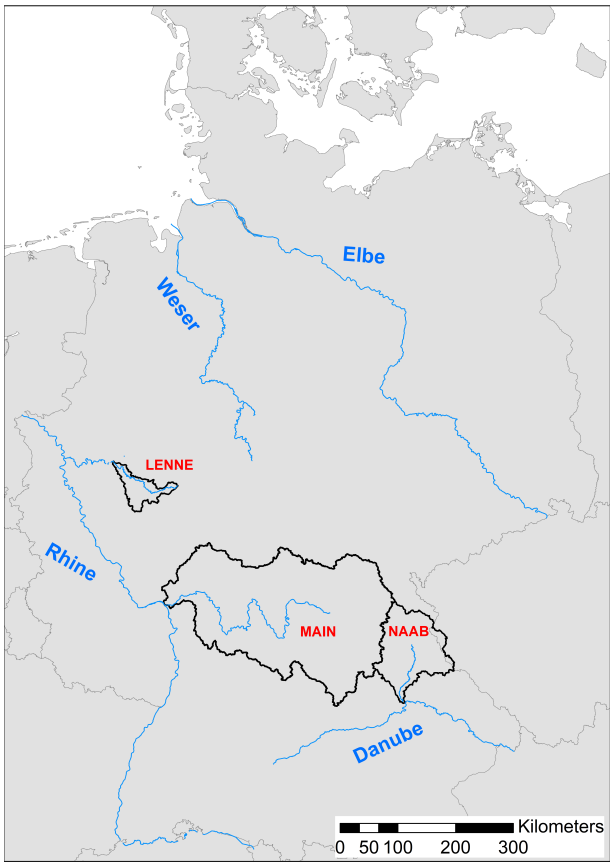

Figure 4.1: Location of the three German case study catchments: Main (1), Lenne (2), and Naab (3).

4.3. Results and discussion

4.3.1. Simulation for clarithromycin

Figure 4.2 shows predicted mean environmental concentrations (PEC), in the whole river basin in form of a color-coded map. This provides a quick overview of the spatial distribution of expected concentrations in the whole watershed and allows for easy identification of river segments with elevated concentrations. The environmental quality standard (EQS) of 130 ng/L for clarithromycin defined in the EU Water Framework Directive (WFD) (Carvalho et al., 2015) is only exceeded in a few small creeks with mean concentrations of up to 187 ng/L (red segments marked by circles in Figure 4.2).

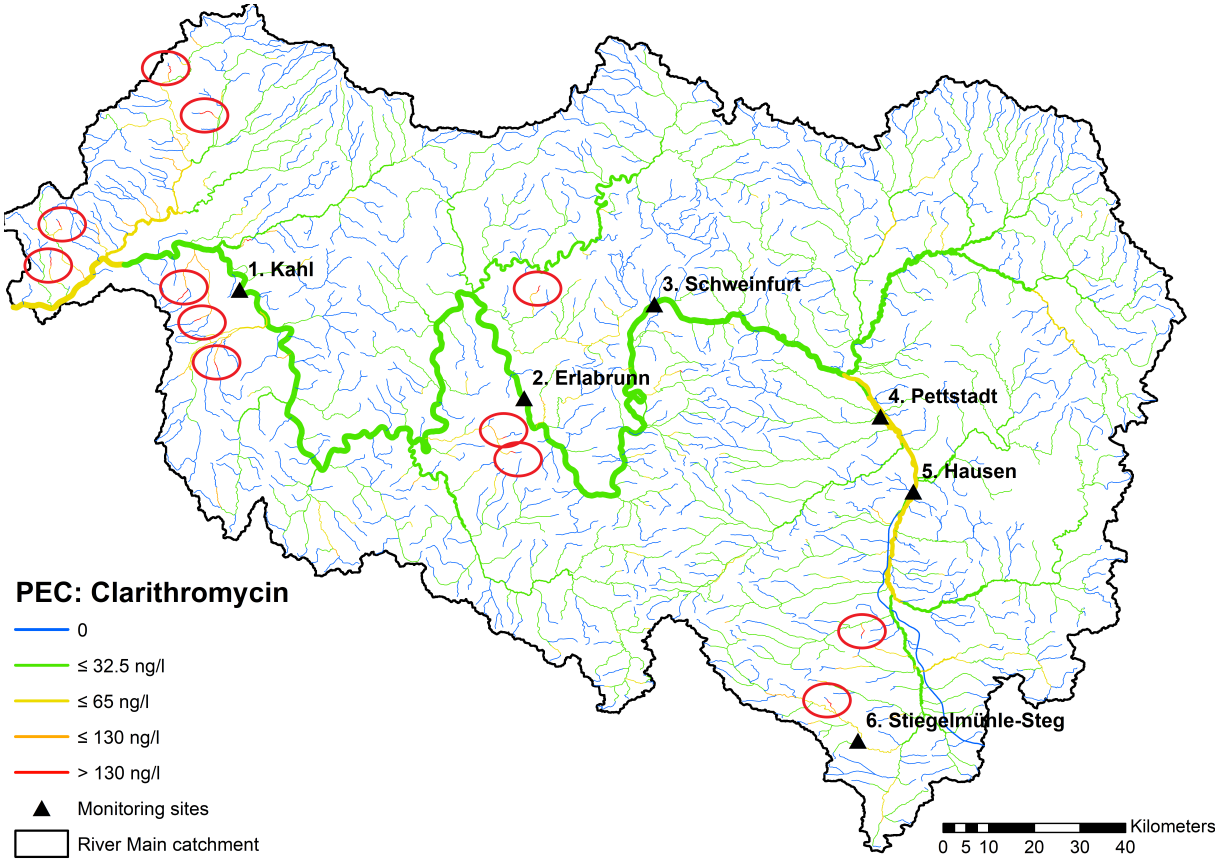

Figure 4.2: Color-coded map of average clarithromycin concentrations in the Main catchment predicted by GREAT-ER; hot spots (sites with highest concentrations) are highlighted by red circles; the six monitoring sites are marked as black triangles.

The EU Commission Directive 2009/90/EC (European Union, 2009) specifies that an exceedance of EQS is incurred when the mean value of all measurements is above this threshold value. From the simulation results, it can be concluded that the majority of the river network will meet this regulatory criterion. Nevertheless, due to the large variability of river flows, concentrations may occasionally exceed the EQS at more sites even

when mean values are below (Ort et al., 2010a). This can be investigated using the results of the probabilistic simulation. The probability distribution represents the expected variation of concentrations over time due to discharge fluctuations and input parameter uncertainties. Comparison with monitoring data was performed at six sites (locations shown in Figure 4.2), for which multiple clarithromycin measurements were available (see Figure 4.3). These sites cover a wide range of average river flow in the catchment going from 2 m^3/s (site 6) up to more than 200 m^3/s (site 1). Figure 4.3 demonstrates that the range spanned by the 10^{th} and 90^{th} percentile of simulated concentrations (displayed in grey) well represents the temporal variability of the monitoring data points at the six sites. At least 80% of the data points are within the respective probability range.

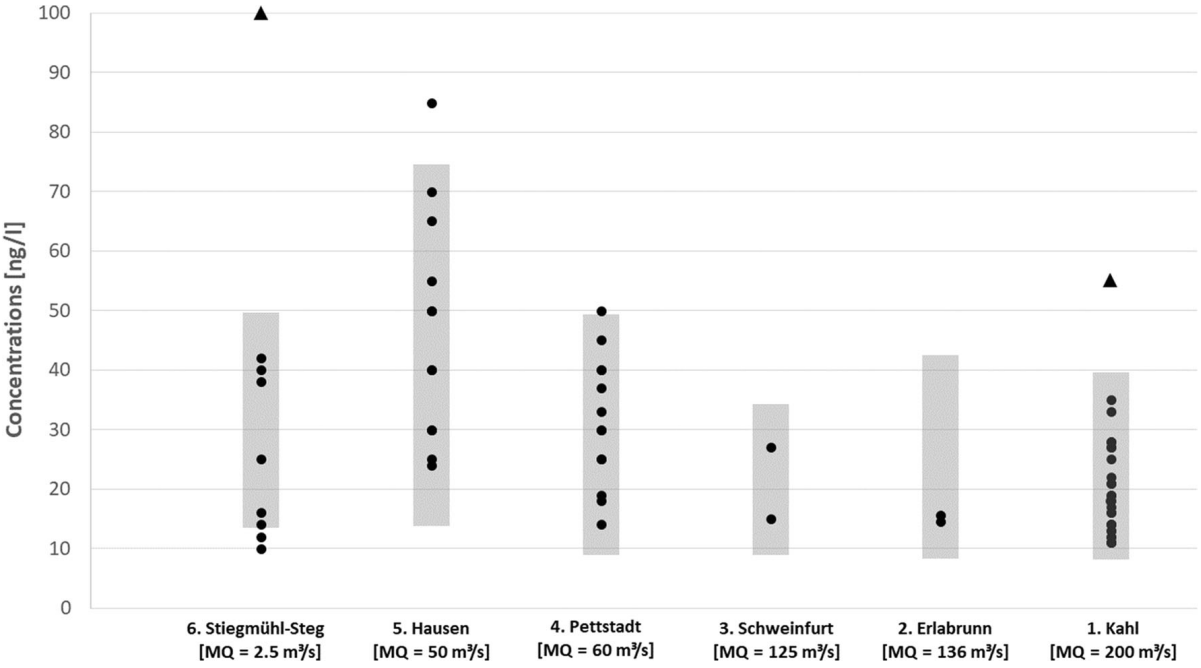

Figure 4.3: Comparison of clarithromycin measurements taken between 2010 and 2017 at the monitoring sites 1–6; sorted according to MQ; marked in grey the 10^{th}-to-90^{th} percent interval of 10 000 simulations runs. Two outliers according to Dean-Dixon test ($p < 0.01$) are marked with a triangle.

On top, the Dean-Dixon test (Dean and Dixon, 1951) for small samples ($n < 30$) identified the two extremely high data points at sites 1 and 6, respectively, as outliers at a significance level of $p = 0.01$. The high concentration value of 100 ng/L at site 6 (Stiegmühl-Steg) may be explicable by specific temporal emissions due to the occurrence of combined sewage overflow (CSO) events. In the sampling period, intense precipitation in the area was recorded resulting in high flow rates approximately 50% above annual mean flow. It could well be that the water sample was affected by a recent CSO event having introduced large amounts of untreated wastewater. Consequently, emission loads

of clarithromycin may have temporally jumped up even overcompensating the dilution effect by the higher flow rate.

4.3.2. Simulation of iopamidol concentrations in the Lenne catchment

X-ray contrast agents such as iopamidol are applied exclusively in hospitals or private doctors' offices for radiology. More than 90% of the applied dosage is excreted via urine within the first 24 h after administration (Duchin et al., 1986). In Switzerland, approximately 50% of X-ray contrast media are administered to stationary inpatients, and 75% of the dosage is already excreted in the urine within 4 h (Weissbrodt et al., 2009). Emissions from stationary treatments will surely enter the wastewater cycle at the location of medicinal treatment. We presume that additionally the first urinary excretion of treated non-stationary patients within the 4 h window will also occur at the treatment site so that 87.5% of the total administered dose was emitted there.

For GREAT-ER model simulations, the iopamidol fraction excreted at the site of medicinal treatment (87.5%) was allocated to the eleven hospitals located in the Lenne catchment proportional to the total number of patients treated in the individual hospital. The resulting emission loads are then routed into the receiving sewage treatment plant, since hospitals are not directly emitting their wastewater into the river basin. The remaining emission fraction from prescriptions to non-stationary patients (12.5%) is still considered by the usual per capita approach according to the place of residence principle. This fraction represents the total iopamidol emission from patients after leaving the hospital or private doctor's office and returning home. Figure 4.4 shows the result of the probabilistic simulation (n = 10 000) based on these assumptions (standard scenario).

The simulation results were compared with monitoring data for iopamidol at six locations (M1–M6) provided by the State Agency for Nature, Environment and Consumer Protection, North Rhine-Westphalia for the period from 2009 to 2015. Five sites are located along the Lenne River, while another one (M6) is in a small tributary, which enters the Lenne between M1 and M2. This site had been sampled on purpose to check the possible influence of the nearby hospital. Figure 4.5 (left) shows that the underlying model assumption of evenly distributed per patient consumption in hospitals (standard scenario) does not well reflect the overall situation of iopamidol concentrations in the Lenne basin. It turned out that the standard scenario underestimates the concentrations measured at M6, while data points at M1 were overestimated (see Figure 4.5). At M6, even the 90$^{\text{th}}$ percentile of the simulation (31 µg/L) is below the four data points (46–110 µg/L) in-

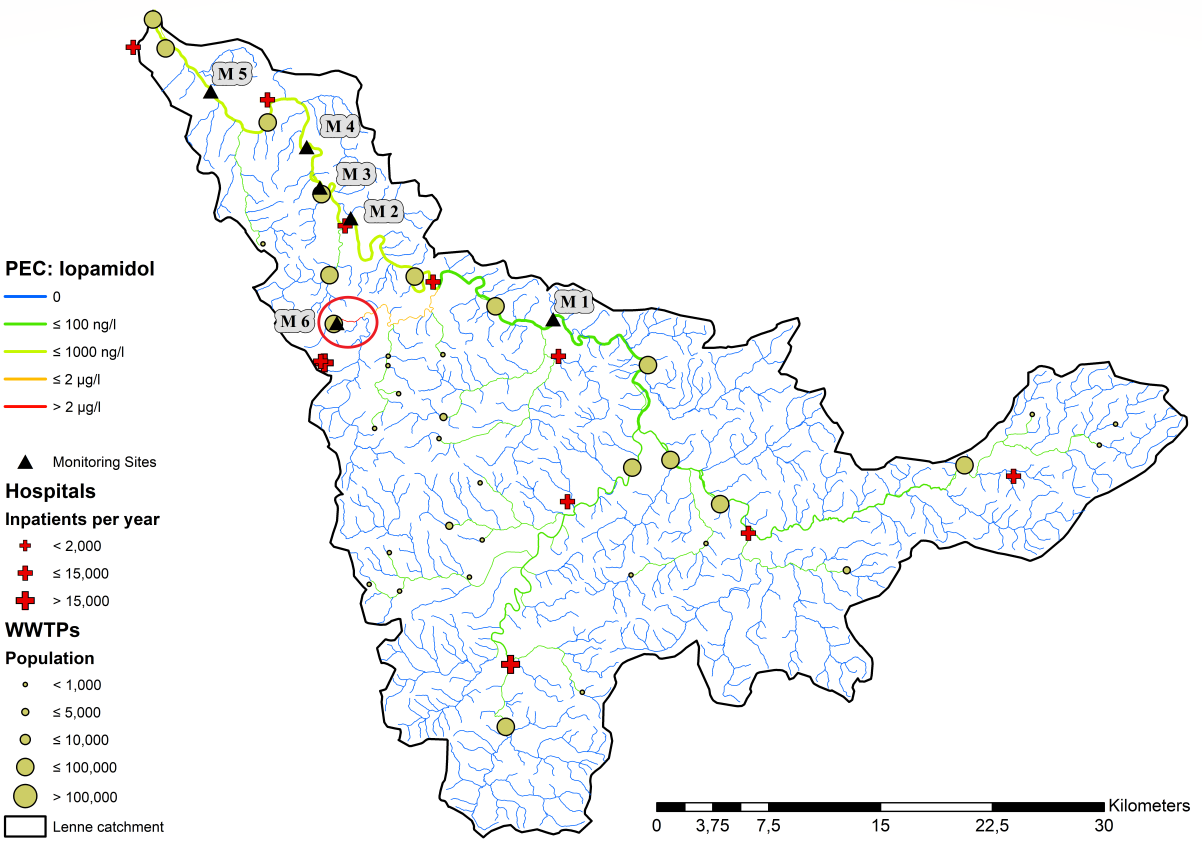

Figure 4.4: Color-coded map of the simulation results of a GREAT-ER model run (n = 10 000) for iopamidol in the Lenne catchment. The six monitoring sites (black triangles) are numbered from M1 to M6.

dicating stronger local influence of the nearby hospital. Further downstream (M2–M5), however, the results of the standard scenario simulation agree well with monitoring data.

It has already been shown that for some pharmaceuticals, the size of the hospitals alone could not always explain observed variations in hospital emissions (Kern et al., 2015; Kuroda et al., 2016). Thus, an overall per patient consumption without taking into account the presence or absence of specialized departments as proposed by Ort et al. (2010b) is not generally applicable. For more realistic local emission estimates, specific information such as department structure, stationary patients, and bed or dosage numbers should be considered if available. Since iopamidol is above all administered in specific radiology departments, the total number of patients may not be the best proxy for estimation of individual hospital emissions. Detailed review then revealed that there is only one hospital in the area, for which a radiology department is officially reported. Most likely, this hospital carries out the majority of radiological treatments with contrast agents relative to the total case numbers per year, as none of the other hospitals in the area is specialized in this field. Thus, for a second scenario, iopamidol emissions from hospitals were

individually adjusted to increase the degree of realism in the model assumptions: The receiving WWTP of the respective hospital with radiology department was now loaded with an above average fraction of the iopamidol emissions, while the other hospitals' contributions were decreased accordingly in order to keep the total emission constant. Before the adjustment, iopamidol emissions from hospitals were evenly distributed depending on their size (number of beds and patients). In the adjusted scenario, the single hospital with the radiology department is assumed responsible for 90% of the iopamidol hospital emissions (79% of overall emission). WWTP emissions from diffuse excretion away from the treatment location remained unchanged at 12.5% of total emissions, since reallocation of hospital contributions does not effect this number.

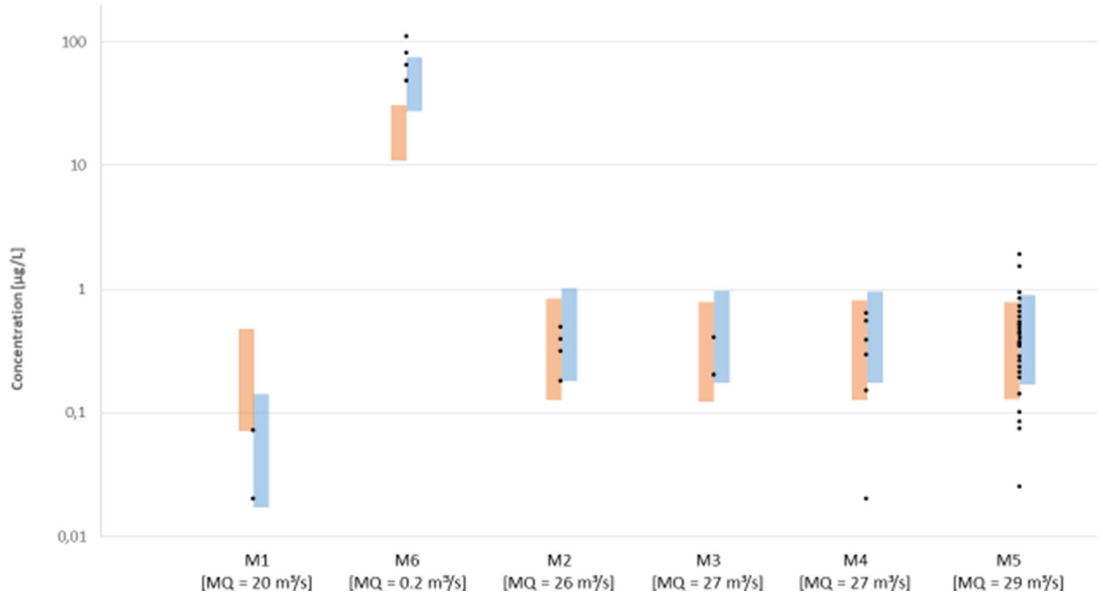

Figure 4.5: Comparison of the 10^{th}-to-90^{th} percent predicted concentration intervals of two probabilistic simulations (each n = 10 000). On the left (orange): interval for the standard scenario. On the right (blue): simulation with consideration of local hospital consumption patterns. Monitoring sites M1—M6 are arranged according the flow path of the Lenne; M6 is integrated according to the position of the tributary.

Figure 4.5 shows simulated concentrations of iopamidol for the two scenarios compared to measured data. The spatial redistribution of iopamidol hospital emissions in the model leads to a much better agreement with monitoring data as compared to the standard scenario at M1 and M6 (Figure 4.5, right), while further downstream (M2–M5), the previous good agreement persists. The model thus allows for consideration of local impacts of hospitals on surface water concentrations for specific pharmaceuticals, while the regional evaluation is only marginally affected. The analysis for iopamidol in the Lenne basin demonstrates that substances predominantly applied in large amounts at hospitals or

private doctor's offices experience a shift in their spatial concentration distribution that may locally be dependent on the presence or absence of specific medicinal departments.

4.3.3. Simulation for ethinylestradiol in the Naab catchment

EE2 was chosen as exemplary compound, because it was on the first WFD watch list (2013) and remained part of the second edition (2018). Although extensive monitoring data have been already collected across Europe, the informative value of the data is still low due to the insufficient limit of quantification (LOQ) of the analytical methods. Only half of the responsible countries were able to quantify EE2 concentrations in the range of the EQS or below (Loos et al., 2018). This is where GREAT-ER simulations can be supportive, since for EE2, the model provides the sole possibility to get a comprehensive picture of the expected concentration range in a whole river basin even when concentrations are below the LOQ.

The standard scenario representing the predicted status quo of average EE2 concentrations in the Naab catchment is displayed on the left-hand side of Figure 4.6. The map reveals that EE2 concentrations in most of the river reaches do not exceed the currently proposed EQS of 35 pg/L (Loos et al., 2018). Moreover, only 65 km of the 2077 km flow length in the Naab basin downstream of WWTPs is predicted to exhibit EE2 concentrations detectable with the standard analytical procedures. Thus, comprehensive exposure assessment by monitoring cannot be achieved for EE2.

It is also seen that concentrations are highest in small creeks receiving wastewater from one of the 102 small treatment plants serving less than 1 000 inhabitants (marked as small green dots in Figure 4.6) with unfavourable dilution ratios. GREAT-ER provides a valuable tool to support authorities in decision-making by *a priori* simulation of the effect of mitigation measures. Therefore, we investigated the effect of a common strategy in the implementation process of the WFD in Germany, namely, re-routing of wastewater from these small WWTPs to the closest treatment plant with higher capacity (e.g. Ministry of Environment and Consumer Protection, State of Saarland, 2018; Ministry of the Environment, Climate Protection and the Energy Sector Baden-Württemberg, 2017). This closest distance boundary condition has been selected to minimize the length of additional sewer pipes for re-routing.

The result of this management scenario is shown in Figure 4.6 (right) as relative comparison with the standard scenario. For river reaches displayed in green, PEC values in the action scenario are lower by at least 5% compared to the reference (improvement), while red river parts exhibit higher values (deterioration). Concentration changes of less than ±5% are regarded insignificant and thus marked gray.

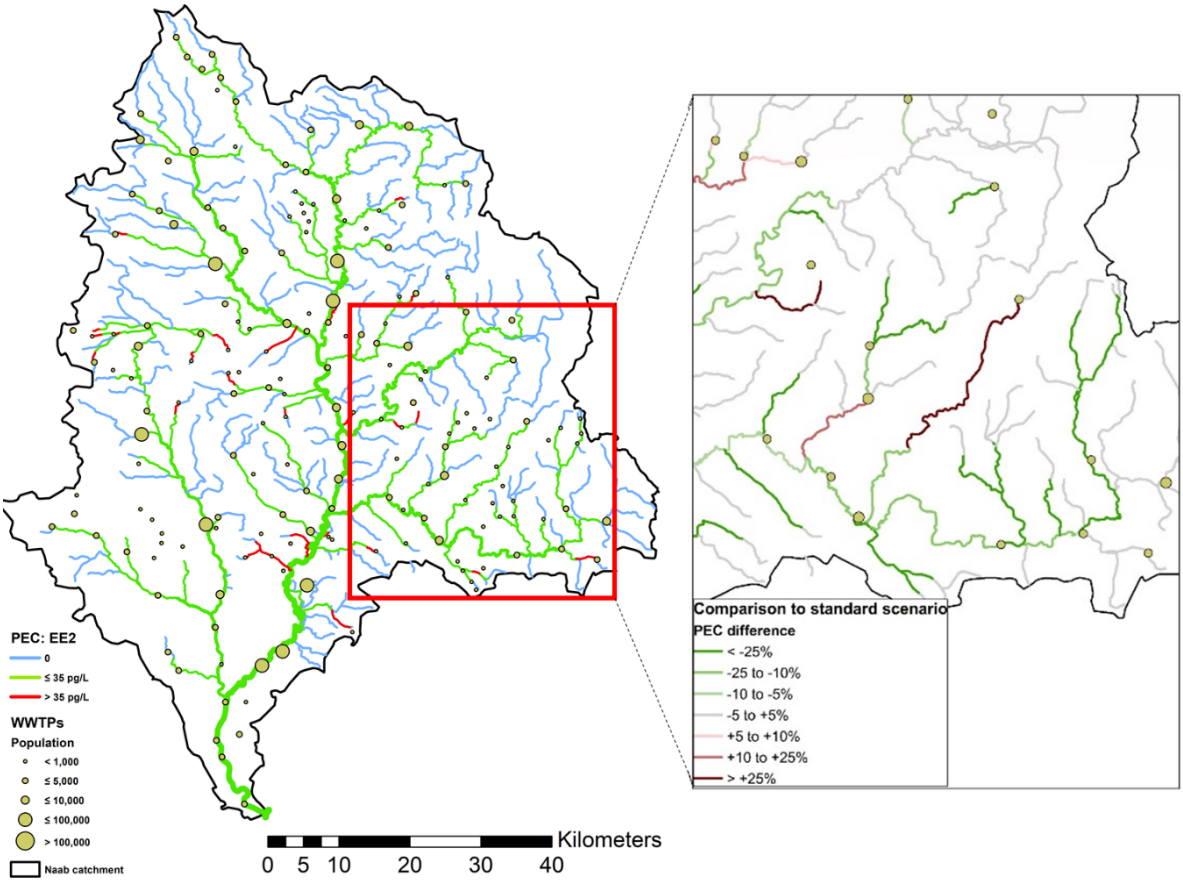

Figure 4.6: Left panel: PEC/EQS standard scenario. Right panel: relative change in PEC between action scenario and standard scenario for an exemplary area in the Naab catchment

In total, lower concentrations are predicted for 655 km flow length (32%) after re-routing, while only 91 km of the river system shows an increase in concentration of more than 5%. 6.1 km is now predicted to be above the EQS where there was no exceedance before, while 38.9 km is now below, resulting in a net relief of 32.8 km in sum. This is a direct consequence of the closest distance boundary condition. In the action scenario, redirection of wastewater does not always occur strictly downstream, because the closest larger treatment plant was sometimes located in another tributary's sub-basin. In this case, water managers would have to evaluate different alternatives to find the best compromise between cost and effect. This case study demonstrates how the GREAT-ER model can support them to do so. In the first step, it provides information about the actual exposure situation (status quo) which allows for deciding whether there is a need for action at all. In the second step, the expected effect of selected measures can be evaluated in order to allow for implementing the most promising strategy taking into account cost-benefit considerations. In the case of EE2, GREAT-ER simulations predict mean concentrations in the Naab basin mostly below the current EQS so that immediate action does not seem to be necessary.

4.4. Conclusions

The geo-referenced steady-state model GREAT-ER simulates the spatial concentration distribution under the assumption of steady state for specific boundary conditions. It was shown that probabilistic simulations considering natural variability of river flow and/or uncertainty of model parameters well predict the expected range of concentrations. We conclude that exposure assessment in river basins should not solely rely on a restricted number of monitoring data but make use of the complementary GREAT-ER model approach.

However, the general assumption of more or less evenly distributed emission patterns does not hold true for pharmaceuticals administered in large fractions in hospitals or private doctors' offices. While this does not largely affect exposure assessment on the regional scale, local assessment may fail for such compounds if the flow path of hospital wastewaters is not explicitly considered in the model representation. Exposure and risk assessment for micropollutants at low concentrations in the range of the limit of detection constitutes a particular challenge. A prominent example for this dilemma is EE2 due to its low exposure concentrations and the low EQS value proposed. While in such cases monitoring alone is not sufficient for basin-wide exposure assessment, this can be achieved with the support of the GREAT-ER model.

An essential part of the GREAT-ER software is the ability to create and analyse specific action scenarios. These features can be used for *a priori* assessment of measures on the catchment scale. For example, re-routing of wastewater from decentralized small WWTPs to larger ones has been shown to provide an option for improvement of the water quality in small creeks with unfavourable dilution factors.

This may be all the more important as the EU recently has run so-called "fitness checks", assessing whether EU Directives are fit for purpose by examining their performance. The WFD was checked aside the Environmental Quality Standards Directive, the Groundwater Directive, and the Floods Directive (European Comission, 2019). While this fitness check states that in Germany, the implementation of the WFD has led to an improvement of the state of numerous waters and the knowledge on pollutant loads and water quality could be increased considerably, it adds that most of Germany's water bodies will not achieve the 2 027 targets (Vermeulen et al., 2019). We conclude that complimentary use of targeted monitoring and geo-referenced modeling constitutes a promising option to save time and money while completing these tasks.

4.5. Acknowledgements

Open Access funding enabled and organized by Projekt DEAL. This work was partly supported by the Bavarian Environmental Agency (LfU) and the State Agency for Nature, Environment and Consumer Production North Rhine-Westphalia (LANUV).

Original publication: Environmental Toxicology and Chemistry **41**(3): 648–662 (2021)
available at http://dx.doi.org/10.1002/etc.5062

Ecological risk assessment of pharmaceuticals in the transboundary Vecht River (Germany and The Netherlands)

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Abstract

Millions of people rely on active pharmaceutical ingredients (APIs) to prevent and cure a wide variety of illnesses in humans and animals, which has led to a steadily increasing consumption of APIs across the globe and concurrent releases of APIs into the environment. In the environment, APIs can have a detrimental impact on wildlife, particularly aquatic wildlife. Therefore, it is essential to assess their potential adverse effects to aquatic ecosystems. The European Water Framework Directive sets out that risk assessment should be performed at the catchment level, crossing borders where needed. The present study defines ecological risk profiles for surface water concentrations of 8 APIs (carbamazepine, ciprofloxacin, cyclophosphamide, diclofenac, erythromycin, 17α-ethinylestradiol, metformin, and metoprolol) in the Vecht River, a transboundary river that crosses several German and Dutch regions. Ultimately, 3 main goals were achieved: 1) the geo-referenced estimation of API concentrations in surface water using the geography-referenced regional exposure assessment tool for European rivers; 2) the derivation of new predicted no-effect concentrations for 7 of the studied APIs, of which 3 were lower than previously derived values; and 3) the creation of detailed spatially explicit ecological risk profiles of APIs under 2 distinct water flow scenarios. Under average flow conditions, carbamazepine, diclofenac, and 17α-ethinylestradiol were systematically estimated to surpass safe ecological concentration thresholds in at least 68% of the catchment's water volume. This increases to 98% under dry summer conditions.

5.1. Introduction

The discovery and manufacture of active pharmaceutical ingredients (APIs) have prompted human and veterinary medicine to a modern era. Many health care and agriculture food production systems around the globe rely on APIs to prevent and cure a wide variety of illnesses in humans and animals, which has led to a sustained consumption of them (Klein et al., 2018). Next to the benefits of APIs, their widespread use has also led to unintended consequences such as antimicrobial resistance (Hernando-Amado et al., 2020; Young, 1993) and environmental pollution (aus der Beek et al., 2016). The occurrence of APIs in the environment can have detrimental impacts on wildlife (Jobling et al., 1998; Saaristo et al., 2018; Shultz et al., 2004). To guarantee a good surface water quality, it is essential to assess potential adverse effects of APIs to aquatic ecosystems. The corresponding legal framework comprises the European Union's Water Framework Directive (European Union, 2000) and the Priority Substances Directive (European Union, 2008).

These directives impose the protection of water resources on European Union member states, for example, by defining environmental quality standards (EQSs) for 45 priority substances. However, none of these substances is an API. Instead, a limited set of APIs is covered in a biennial watch list of water pollutants that should be carefully monitored because of insufficient monitoring data and concerns about their ecological impact. The Water Framework Directive calls for a basin approach, moving away from national risk assessments (Coppens et al., 2015; Vissers et al., 2017) and complementing it with more detailed, in some cases transboundary, catchment-wide risk assessments. Determination of the chemical status of a surface water within the context of the Water Framework Directive relies on the quantification of risk by integrating exposure and effect assessments.

Exposure assessment can be based on measured environmental concentrations (MECs), predicted environmental concentrations (PECs) using chemical fate models or a combination of both. In the past 30 years, a variety of models have been developed to derive PECs for chemicals, such as ePiE (Oldenkamp et al., 2018), iStream (Kapo et al., 2016), a contaminant fate model (Grill et al., 2016), PhATE (Anderson et al., 2004), STREAM-EU (Lindim et al., 2016), GLOBAL-FATE (Font et al., 2019), and the geography-referenced regional exposure assessment tool for European rivers (GREAT-ER) (Feijtel et al., 1997; Kehrein et al., 2015; Lämmchen et al., 2021c), varying in complexity and geographical and temporal resolution. The concentration gradient along a watercourse is highly dependent on local socioeconomic and environmental factors. Therefore, the degree of access to detailed local data (e.g. pharmaceutical consumption patterns) and spatiotemporal information (e.g. seasonal hydrological landscape) is an important driver for the accuracy of exposure models at the catchment level (Font et al., 2019; Oldenkamp et al., 2018; Tiedeken et al., 2017).

A comprehensive effect assessment requires extensive ecotoxicological information to derive safe concentration thresholds for aquatic ecosystems, for example, predicted no-effect concentrations (PNECs) or EQSs. To optimize the accuracy of the assessment, it is common practice to gather all available toxic effect data on a substance and select an extrapolation method that matches the available data. Therefore, the estimation and accuracy of useful PNECs is highly dependent on up-to-date ecotoxicological data and requires continuous revision to accommodate new evidence.

Riverine ecological assessments conducted in Europe and elsewhere have recurrently found APIs and other emerging pollutants to pose a potential risk to freshwater biota (Gómez-Canela et al., 2019). A main obstacle to modeling studies of API residues in transboundary catchments is the restricted access to detailed national and regional API-specific consumption data (Tiedeken et al., 2017). Additional obstacles include different national and regional water management strategies, diverse wastewater treatment efficiencies, the

heterogeneity of the landscape, seasonal variation in environmental conditions, and variable demographics (Popelka and Smith, 2020). The main aim of the present study was to construct ecological risk profiles for surface water concentrations of 8 environmental residues of APIs in the European transboundary Vecht River, a river that crosses several German and Dutch regions. Firstly, an exposure assessment was performed by the applying the geo-referenced model GREAT-ER, which has a good track record for predicting pharmaceutical PECs in river catchments (Aldekoa et al., 2013; Alder et al., 2010; Archundia et al., 2018; Caldwell et al., 2019; Capdevielle et al., 2008; Cunningham, 2008; Hanamoto et al., 2013; Hannah et al., 2009; Schowanek and Webb, 2002; Zhang et al., 2015). Secondly, an effect assessment was performed based on existing ecotoxicological information. This information was used to determine PNECs by incorporating recent test results. Finally, PECs and PNECs were coalesced into ecological risk quotients (RQs) throughout the Vecht River network under 2 distinct water flow condition scenarios. This helps improve our understanding of the risk posed by APIs to local freshwater communities and advances the ability to evaluate and prioritize potential (local) mitigation strategies before their implementation by competent authorities (Government of the Netherlands, 2019).

5.2. Materials and methods

5.2.1. Pharmaceuticals

Ecological risks were assessed for 8 selected APIs (Table 5.1). These represent only a subset of APIs detected in the Vecht River catchment (data not shown). The selection covers a wide range of consumption patterns, therapeutic classes, chemical properties, and levels of data availability (Appendix B).

Table 5.1: Names, Chemical Abstracts Service numbers, Anatomical Therapeutic Chemical codes, and therapeutic classes of the 8 active pharmaceutical ingredients assessed in the present study. API = active pharmaceutical ingredient; CAS = Chemical Abstracts Service; ATC = Anatomical Therapeutic Chemical.

API	CAS no.	ATC code	Therapeutic class
17α-ethinylestradiol[a]	57-63-6	G03CA01	Sex hormones
Carbamazepine[c]	298-46-4	N03AF01	Antiepileptics
Ciprofloxacin[b]	85721-33-1	J01MA02	Antibacterials
Cyclophosphamide	50-18-0	L01AA01	Antineoplastics
Diclofenac[a]	15307-86-5	M01AB05	NSAID
Erythromycin[a]	114-07-8	J01FA01; QJ01FA01[d]	Antibacterials
Metformin[c]	657-24-9	A10BA02	Antidiabetics
Metoprolol	37350-58-6	C07AB02	Beta-blockers

[a] Substance excluded from the watch list under the Water Framework Directive (Cortes et al., 2020).

[b] Substance included in the watch list under the Water Framework Directive (Cortes et al., 2020).

[c] Candidate substance suggested by individual member for inclusion for the next watch list under the Water Framework Directive (Cortes et al., 2020).

[d] Substance used in human and veterinary medicine.

5.2.2. Case study area

The study area comprises the catchment area of the German and Dutch transboundary Vecht River, a tributary of the Dutch IJssel River. The area is under the influence of diverse anthropological stressors (e.g. treated wastewater emissions, water level control via pumps and locks) (Lämmchen et al., 2021b; Lulofs and Coenen, 2007; Wöhler et al., 2020). The catchment extends over an area of approximately 6 100 km^2. The total length of the Vecht River itself amounts to 167 km, of which approximately 107 km are located in Germany.

The German part of the catchment is located in the western part of Lower Saxony and in small sections of North Rhine- Westphalia, comprising the smaller part of the total catchment area with a share of 1 800 km^2 (Figure 5.1). In Germany, the Vecht is a medium-sized river (long-term annual average flow of approximately 18.5 m^3/s at the

German-Dutch border) with many small tributaries, for example, the Steinfurter Aa and the Dinkel.

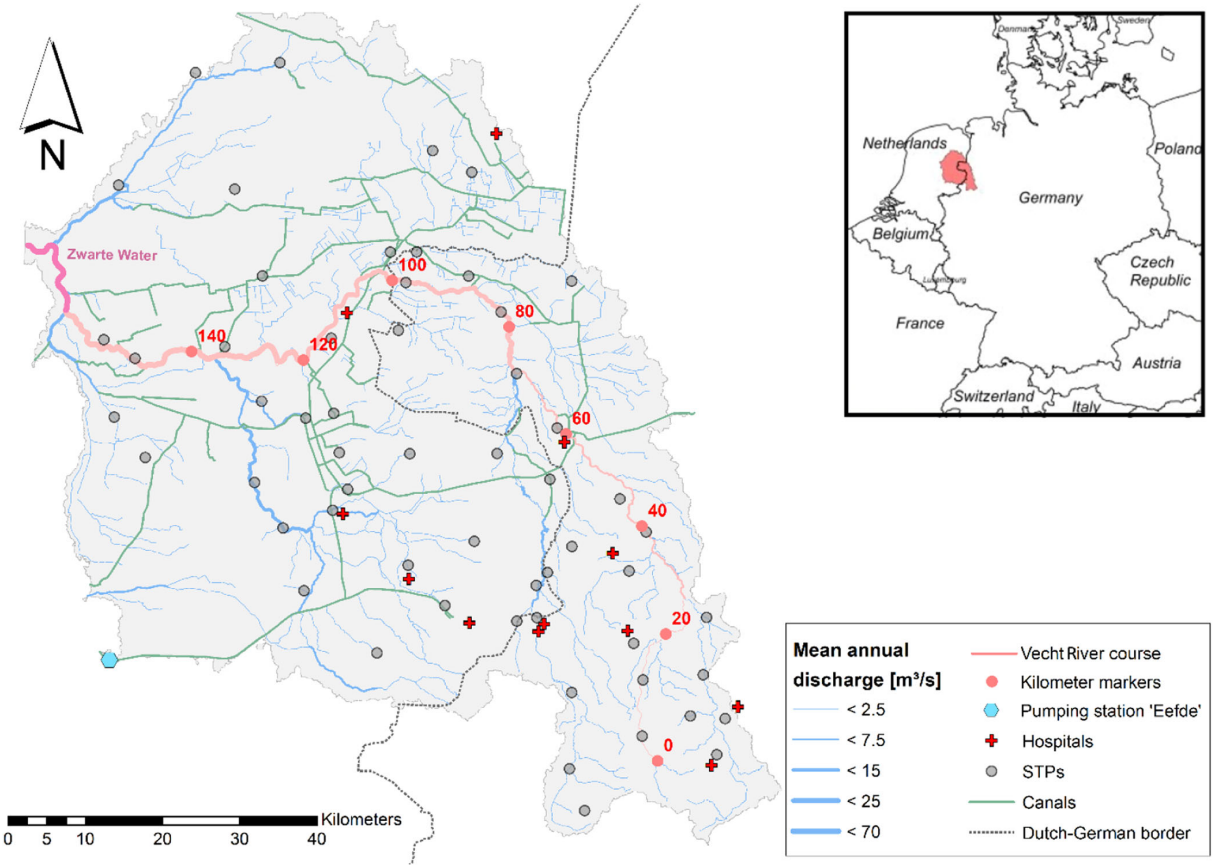

Figure 5.1: Vecht River basin. Kilometer markers start at the confluence of the Vecht tributaries Burloer Bach and Rockeler Mühlenbach. STPs = sewage treatment plants.

The river system is still in an almost natural state in the German regions (Lulofs and Coenen, 2007), with a few canals (e.g. Ems-Vecht Canal and the Nordhorn-Almelo Canal) having negligible influence on river flow. The German part is less densely populated (160 cap/km^2) than the Dutch part (260 cap/km^2) because only small towns such as Nordhorn and Gronau (approx. 50 000 inhabitants) are located in this area. In total, emissions from approximately 400 000 inhabitants connected to 25 sewage treatment plants (STPs) enter the German Vecht. In addition, the wastewater of 6 hospitals with approximately 1 200 beds in total is treated by the STPs.

Approximately 4 300 km^2 of the transboundary catchment is located in the Netherlands, namely in the provinces of Overijssel and Drenthe. This part of the catchment is highly influenced by anthropogenic activities, which resulted in canals, sluices, pumps, and river straightening (Lämmchen et al., 2021b; Lulofs and Coenen, 2007). Larger cities with

more than 100 000 inhabitants are Enschede, Zwolle, and Emmen. In total, more than 1 000 000 inhabitants are connected to 32 STPs, as are 7 hospitals with approximately 2 000 beds in total. The Zwarte Water River, a short prolongation of the Vecht River and an inflow of the Zwarte Meer Lake, was integrated into the model representation.

5.2.3. Environmental exposure assessment

The GREAT-ER model was used to predict environmental concentrations of the 8 case study APIs. The GREAT-ER model was originally developed to predict spatially explicit stationary exposure concentrations of "down-the-drain" chemicals in surface waters at the catchment level (Feijtel et al., 1997). The model has been successfully applied to various chemicals in different European catchments (Aldekoa et al., 2013; Alder et al., 2010; Hüffmeyer et al., 2009; Kehrein et al., 2015). A detailed description of the functions of the model and its latest extensions can be found in Kehrein et al. (2015) and Lämmchen et al. (2021c). The model mainly consists of 3 components: the hydrological network, the emission model, and the fate model. The hydrological network is the centerpiece of the GREAT-ER model. The water network is discretized into river segments with a length of up to 2 km. Each segment carries a property vector that is used to calculate the chemical's fate and concentration.

Exposure scenarios

The steady-state model GREAT-ER represents a static hydrological situation over time. Two different scenarios were set up for the hydrological network, a low-flow condition scenario (mostly dry periods in summer) and an average-flow condition scenario (Table 5.2). This allows for considering the effect of the change of flow directions in some parts of the network during dry periods caused by pumping systems in the Dutch canals (Lämmchen et al., 2021b).

Table 5.2: Characteristics of the simulated low-flow and average-flow condition scenarios.

	Dry summer scenario	Average condition scenario
Applicability	Dry periods without rainfall between June and September	Humid periods throughout the year
Flow rate at the border (m^3/s)	2.82	18.5
Flow rate at the Zwarte water (m^3/s)	11.31	63.45
Flow velocity at the border (m/s)	0.22	0.6
Flow velocity at the Zwarte Water (m/s)	0.33	0.85
Pumping activity	Yes	No
Pumping description	120 d/yr between March and October (the Netherlands)	—
Pump power	"Eefde" (Twente Canal; m^3/s) 1.6 (mean), 14 (maximum)	—
Changes in flow direction	Yes: Twente Canal, Zijkanaal Almelo, Canal Almelo-De Haandrik and several emerging smaller canals	No

Model parameterization

A key input parameter is the consumption of APIs in the investigated area. It is well known that consumption patterns sometimes vary between countries and regions, which holds true for some of the investigated compounds in the Netherlands and Germany (Table 5.3). Regional sales data for the Vecht catchment from 2017 were acquired for the regions in Germany and the Netherlands from IQVIA Commercial GmbH & Co. OHG (IQVIA, Frankfurt am Main, Germany, unpublished data) and the Dutch Foundation for Pharmaceutical Statistics (SFK, The Hague, Netherlands, unpublished data) at the postcode level (Table B.1). Data include pharmacy sales but not the amount dispensed in hospitals, nursing homes, or by general practitioners. Drugs sold over the counter are included in the German data set but not in the Dutch data set. Annual prescription data were divided by the population number in the respective area, resulting in average per capita consumption values (Table B.1). The contribution of hospitals was considered in terms of a per-bed application. This number was different for the 2 countries and was estimated from available prescription data of selected hospitals on both sides of the border (Table B.1).

Table 5.3: Relative percentage differences of prescribed per capita pharmaceutical masses in the Vecht River basin regional area, Germany and the Netherlands.

	Regional-to-national (%)		Germany-to-the Netherlands (%)	
	Germany	Netherlands	Within region	Between countries
17α-ethinylestradiol	12	-2	-75	-78
Carbamazepine	-4	16	2	25
Ciprofloxacin	9	10	27	28
Cyclophosphamide[a]	33	n.a.[b]	n.a.[b]	n.a.[b]
Diclofenac	-2	-2	183	183
Erythromycin	56	-13	1 594	853
Metformin	-14	6	-26	-9
Metoprolol	-8	22	-10	20

[a] Cyclophosphamide is restricted to clinical use. The Dutch Foundation for Pharmaceutical Statistics only collects domestic pharmaceutical consumption. Therefore, no cyclophosphamide is recorded for the Netherlands.

[b] n.a. = not applicable.

Emission loads into the sewer system of an STP were estimated by multiplying the per capita and per bed application rates with the number of connected inhabitants or hospital beds, respectively. Because most APIs are metabolized after uptake, only the excreted fraction was considered (Table B.2). Metabolites such as glucuronides, which react back to the parent compound after release into the sewer, were also included (Heberer and Feldmann, 2005).

A fraction of the excreted amount is removed during wastewater treatment in STPs. In the Vecht River catchment, all STPs are equipped with biological treatment with no additional stage for further elimination of micropollutants such as ozonation, ultrafiltration, or activated charcoal filtration. Although removal efficiencies may depend on the specific operating conditions (Verlicchi et al., 2012), equal removal efficiency for each API in all STPs was assumed.

From a comprehensive literature search, removal efficiencies determined in STPs equipped with biological treatment collected as composite samples (> 24 h) were used to calculate median values for the model simulations (Table B.4).

The estimated load in treated effluents is routed into the receiving rivers at the respective discharge points. Cumulated loads are propagated through the river network and used to estimate spatially resolved API concentrations (PECs) through division of the load by the respective river flow rate. In addition, the fate model accounts for physicochemical loss processes such as (bio-) degradation, sedimentation, and photolysis. Degradation via hydrolysis and dissipation via volatilization were not accounted for because of their negligible influence on APIs (Patel et al., 2019). A detailed overview of the parameterization of in-stream processes is provided in Table B.5.

Model evaluation

The model performance was evaluated stepwise by comparison of simulation results with monitoring data for selected APIs in STP influents and effluents as well as at selected river sites (Figures 5.2 and 5.3). A comprehensive description of the sampling strategy is provided elsewhere (van Heijnsbergen et al., 2022). A brief overview and details for the chemical analysis are provided in Appendix B (Section B.2 Monitoring campaign).

Two model performance quantitative measures were applied: median symmetric accuracy (ξ) and the symmetric signed percentage bias (SSPB) (Morley et al., 2018),

$$r_i = \frac{x_{i,\text{pred}}}{x_{i,\text{meas}}} \tag{5.1}$$

$$\xi(\%) = 100 \times \left(e^{\text{M}(|\ln r_i|)} - 1\right) \tag{5.2}$$

$$SSPB(\%) = 100 \times \left(e^{|\text{M}(\ln r_i)|} - 1\right) \times \text{sgn}(\text{M}(\ln r_i)\big) \tag{5.3}$$

where r_i is the ratio of the predicted/measured pair (e.g. loads), $x_{i,\text{pred}}$ is the predicted value, $x_{i,\text{meas}}$ is the corresponding value from the measurement data, M is the median function, sgn is the sign function, and i is the index within a subgroup of all predicted/measured pairs for a single compound, scenario, country, sampling site, or a combination of these.

The median symmetric accuracy (Equation 5.2) is a measure of central tendency that is robust to the presence of outliers and resistant to data spanning several orders of magnitude. For the scope of the present study, we consider ξ values up to 100 and up to 200% as indicative of "good agreement" and "acceptable agreement" between measurements and predictions, respectively. Values of $\xi > 200\%$ indicate "poor agreement" between measurements and predictions. A $\xi = 100\%$ indicates that the median of the absolute ratios ($|r_i|$) is 2 (i.e. 50% of predicted values deviate from measured values by less than a factor of 2). The symmetric signed percentage bias (Equation 5.3) can be interpreted similarly to a mean percentage error, but it penalizes underestimation and overestimation

equally. Positive values indicate a tendency to overestimate predictions, whereas negative values indicate a tendency to underestimate predictions. In the present study, absolute values of SSPB up to 50, 100, and 200% were considered as an indication of "small", "medium", and "large" overestimations or underestimations, respectively. Absolute values > 200% were considered "very large" overestimations/underestimations. An SSPB = -50% indicates that the median of relative ratios (r_i) is 50% lower in the predictions

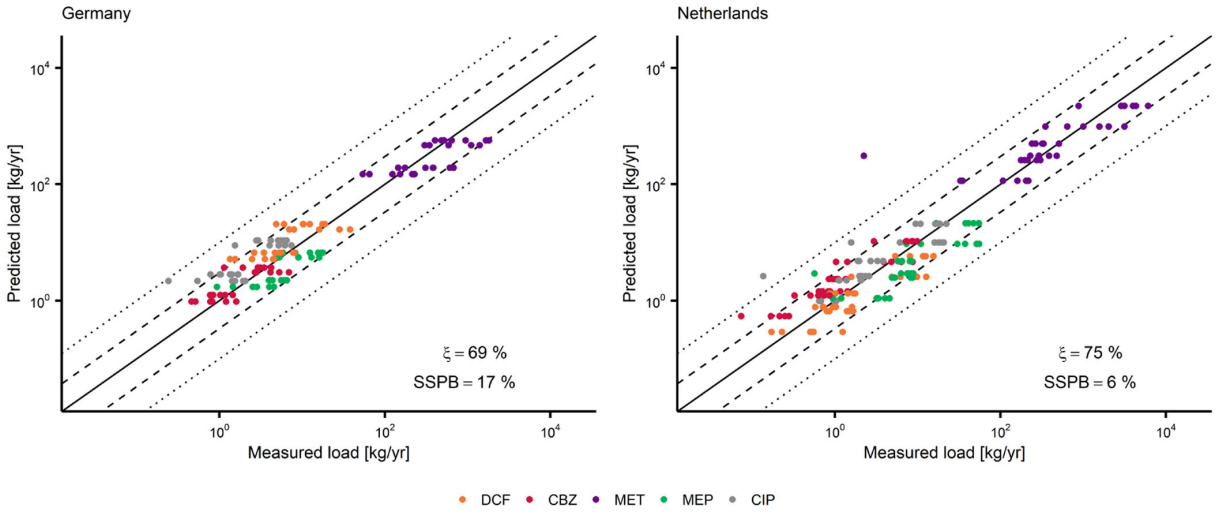

Figure 5.2: Predicted and measured sewage treatment plant (STP) influent loads of 5 pharmaceuticals (with quantification frequency > 90%) in German STPs (n = 125) and Dutch STPs (n = 170). Dashed lines indicate the 1:3 and 3:1 ratios; dotted lines indicate the 1:10 and 10:1 ratios. SSPB = symmetric signed percentage bias; DCF = diclofenac; CBZ = carbamazepine; MET = metformin; MEP = metoprolol; CIP = ciprofloxacin.

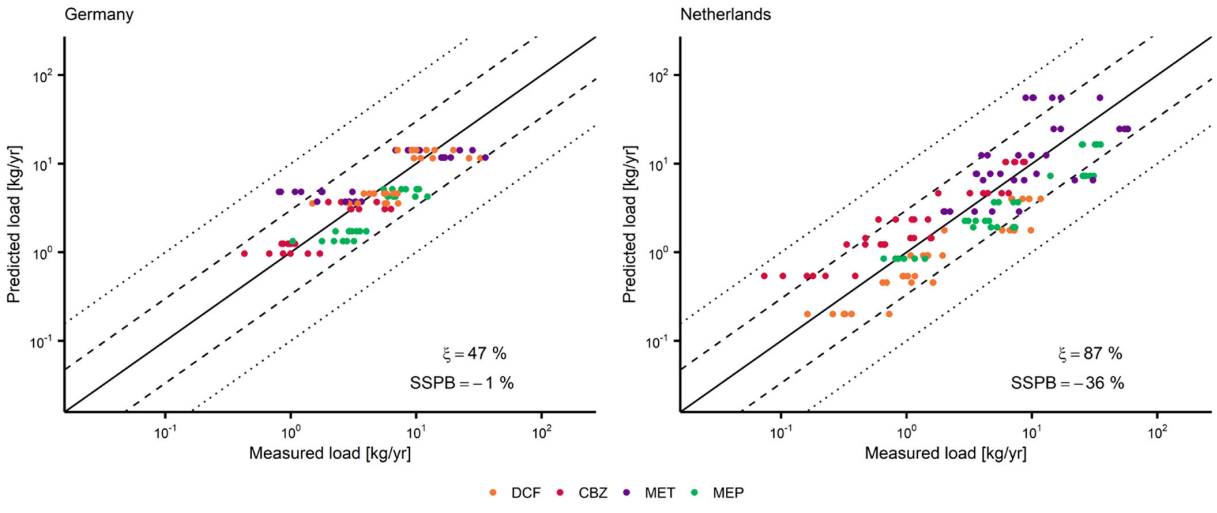

Figure 5.3: Predicted and measured sewage treatment plant (STP) effluent loads of 4 pharmaceuticals (with quantification frequency > 90%) in German STPs (n = 100) and Dutch STPs (n = 132). Dashed lines indicate the 1:3 and 3:1 ratios; dotted lines indicate the 1:10 and 10:1 ratios. SSPB = symmetric signed percentage bias; DCF = diclofenac; CBZ = carbamazepine; MET = metformin; MEP = metoprolol.

compared to measured data. This implies that 50% of the predicted values underestimate the measurements by at least a factor of 1.5. Predictions of STP emissions were evaluated on a load-based approach. Measured concentrations in STP influent and effluent were multiplied with the annual discharge of the corresponding STP and compared to model predictions. The APIs with a quantification frequency $< 90\%$ were evaluated semi-quantitatively. Concentrations below the limits of quantification (LOQ) were processed as LOQ in the evaluation approach because they are expected to be close to the LOQ value as a result of the high quantification frequency.

Surface water PECs were evaluated using the "benchmark" concept, according to Kunkel and Radke (2012), with which concentrations of individual APIs are normalized to the concentration of a conservative tracer or reference. Thereby, river flow variations can be excluded from the evaluation process. Carbamazepine was selected as the conservative reference compound because of its persistence in the environment (Aminot et al., 2016). Benchmark ratios from the monitoring data could only be calculated if the concentration of the reference (carbamazepine) and that of the respective target API were above the LOQ. To provide a reliable baseline for this approach, predicted carbamazepine concentrations were evaluated by comparison with measured concentrations (Section B.3).

5.2.4. Environmental effect assessment

Search strategy

Aquatic ecotoxicity data were compiled without restrictions from the following databases: ECOTOX Knowledgebase (U.S. Environmental Protection Agency, 2019), e-toxBase (Posthuma et al., 2019), Wikipharma (Molander et al., 2009), FASS (Trade Association for the Research-Based Pharmaceutical Industry in Sweden, 2019), iPiESum (Innovative Medicines Initiative, 2019), and the EU WRC report (Johnson and Harvey, 2002). To further supplement collected data, a literature review was performed by searching the Web of Science platform in March 2019 (Table B.11). The search was restricted to publications from 2016 or later to capture information not covered by the other sources. The search returned 233 publications that were fully assessed.

Data extraction and harmonization

All relevant toxicological information referring to the 8 APIs of interest was extracted from the databases. Additional toxicity data were extracted from 40 publications identified in the public literature search. The following relevant information was extracted and compiled: substance name, Chemical Abstracts Service number, taxon, species, life

stage and living compartment of the species tested, toxic effect, exposure type, exposure duration, endpoint type, and endpoint value. This process resulted in an initial database with a total of 11 029 entries (Table 5.4). The data were harmonized to guarantee their consistency and usability, which included harmonizing the names of species, toxic effects, exposure duration and types, end points, and concentration units (Section B.4 Aquatic ecotoxicity data).

Table 5.4: Number of ecotoxicological data entries per source in the database compiled in the present study Source Entries.

ECOTOXbase	6510
Wikipharma	2802
e-toxBase	779
Literature	455
IpiESum	270
EU WRC report	140
FASS	74

Data selection

The information in the database was filtered to obtain only relevant data for analysis. Only aquatic or semiaquatic species were included. Entries referring to terrestrial species, communities, sediment tests with no reported water concentrations, or in vitro tests or with no single species name specified were excluded from the analysis. Only population-relevant endpoints were selected, that is, those which can adversely affect an organism's survival, ability to maintain its population numbers, reproduction, development, growth, or behaviour. Effect endpoints with right/left-censored values (i.e. $<$, $>$, $\leq$, $\geq$) were excluded. Similarly, identical effect entries from the same original source were excluded. Toxicity values for the same species and endpoint but originating from different studies were aggregated by taking the geometric mean weighted by the number studies with identical endpoints. This resulted in a final database containing 169 effect values usable for further analysis.

Data reliability

To ensure that we only included reliable and relevant toxicity studies in our assessment, all studies were assigned a criteria for reporting and evaluating ecotoxicity data (CRED)

score (Moermond et al., 2016). Studies classified as unreliable (R3), unassignable reliability (R4), irrelevant (C3), or unassignable relevance (C4) were excluded from further analysis. We preferably used classification scores from official sources, such as the Dutch National Institute for Public Health and the Environment and the German Environment Agency. Alternatively, the authors (D.J. Duarte, R. Oldenkamp, and A.M.J. Ragas) independently assigned CRED scores to critical studies according to Moermond et al. (2016) after evaluating and discussing any inconsistencies (Table B.12). Exceptionally, experiments on 17α-ethinylestradiol without classifications from official sources were not evaluated because of the extensive number of studies and additional complexity of assessing the quality of ecotoxicological studies testing endocrine-disrupting effects; such an exhaustive assessment was considered beyond the scope of the present study.

PNECs

Two extrapolation methods for the derivation of chronic PNEC values are typically used in effect assessment: the species sensitivity distribution (SSD) and the assessment factor (European Union, 2000, 2006a). According to European Union guidance, an SSD-based PNEC requires a considerable amount of data covering at least 3 trophic levels (primary producers, plant-eating animals, and predators), at least 8 taxonomic groups, and at least 10 effect values (one per species per substance). As for the assessment factor approach, at least one short-term median effective concentration from each of the 3 trophic levels is the minimum requirement. Because the final database did not satisfy SSD data requirements for the derivation of PNECs, only the assessment factor approach was implemented (Table B.15). The estimation of a PNEC using this deterministic approach was done by dividing the lowest effect concentration by an assessment factor, according to the European Union Water Framework Directive guidance for deriving aquatic EQSs (European Comission, 2018). Depending on the available data, this factor varies between 10 and 1 000. A collection of PNEC estimates from the literature and other sources was gathered for comparison (Table B.16).

Ecological risk

Predicted environmental concentrations and PNECs were used to calculate a site-specific RQ associated with each API following the equation,

$$RQ_{s,p} = \frac{\mathrm{PEC}_{s,p}}{\mathrm{PNEC}_p} \tag{5.4}$$

where $RQ_{s,p}$ is the RQ at site s for pharmaceutical p, $\mathrm{PEC}_{s,p}$ [µg/L] is the PEC at site s for pharmaceutical p, and PNEC_p [µg/L] is the PNEC for pharmaceutical p. Evaluation of PNEC exceedance was performed based on the total river volume in the Vecht catchment and for the cumulated flow length of the water bodies in the catchment. Because of the steady-state assumption of the GREAT-ER model, a constant water volume in the system is assumed for each of the scenarios. Pharmaceutical mixture risk was calculated based on the conservative approach of concentration addition following the equation,

$$RI_s = \sum_{i=1}^{n} RQ_{s,p} \tag{5.5}$$

where RI_s is the risk index of a pharmaceutical mixture at site s, $RQ_{s,p}$ is the risk quotient at site s for pharmaceutical p, i is the summation index, and n is the total number of APIs. The concentration addition approach tends to overestimate the mixture risk of dissimilarly acting substances because it assumes a similar noninteractive mode of action of all mixture components. However, there is growing consensus on the pragmatic and precautious utility of this approach in aggregating risks of mixture components (Backhaus and Faust, 2012; European Comission, 2012; Hernandez et al., 2019; Kienzler et al., 2019; Posthuma et al., 2019).

5.3. Results and Discussion

5.3.1. Predicted surface water concentrations

Predicted carbamazepine concentrations were evaluated to provide a reliable baseline for the benchmark approach (Section B). Because carbamazepine is consumed equally throughout the year, evaluation can be performed using all data without differentiation into the 2 exposure scenarios (see above, Exposure scenarios). Figure 5.4 shows an acceptable overall agreement between PECs and MECs (ξ= 106%), with a tendency to being rather overestimated (SSPB = 59%). Approximately 80% of the PEC and MEC data differ by less than a factor of 3, so we conclude that carbamazepine provided a valid baseline for the application of the benchmark approach (Figure B.3).

The quantification frequency of erythromycin and ciprofloxacin in the river samples was <10%. Because all predicted concentrations of these compounds were below the LOQ, qualitative agreement is given. Cyclophosphamide and 17α-ethinylestradiol were not analyzed at all because of the expectation of very low concentrations far below the LOQ. Diclofenac, metformin, and metoprolol concentrations were evaluated separately for the

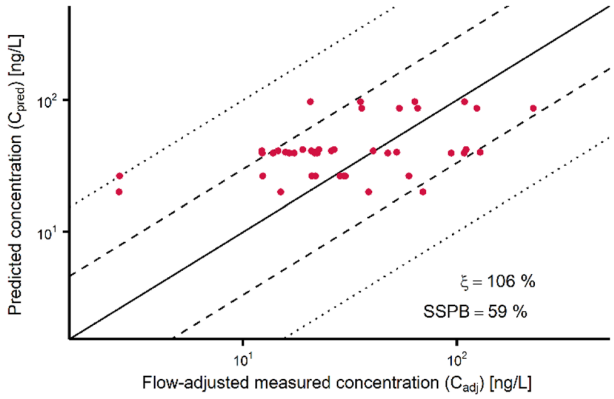

Figure 5.4: Comparison of predicted and measured carbamazepine concentrations in the Vecht catchment (n = 46) at monitoring sites where reliable gauging data of the corresponding sampling day were available (i.e. no change in flow direction, resulting in net flow rates of 0 m^3/s). Measured concentrations were adjusted to the flow rate used in the simulations. Dashed lines indicate the 1:3 and 3:1 ratios; dotted lines indicate the 1:10 and 10:1 ratios. SSPB = symmetric signed percentage bias.

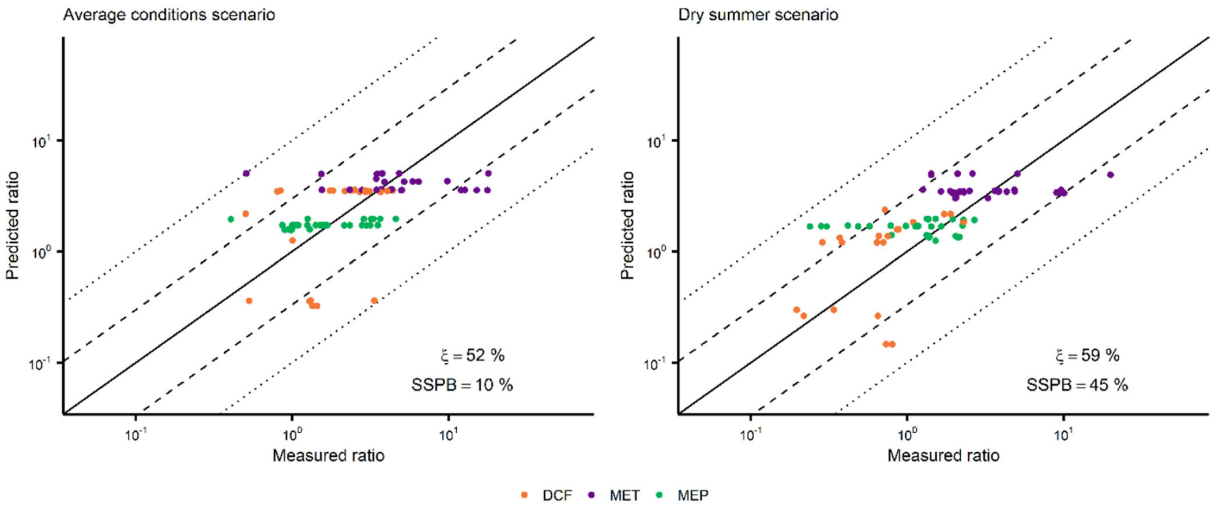

Figure 5.5: Predicted and measured benchmark ratios of 3 pharmaceuticals at monitoring sites in the whole Vecht River catchment (average condition scenario n = 80, dry summer scenario n = 81). Dashed lines indicate the 1:3 and 3:1 ratios; dotted lines indicate the 1:10 and 10:1 ratios. SSPB = symmetric signed percentage bias; DCF = diclofenac; MET = metformin; MEP = metoprolol.

2 exposure scenarios because of obvious seasonal differences (see above, Exposure scenarios). Predicted and measured benchmark ratios agreed well for both the average condition scenario (Scn_{AC}; ξ= 52%, SSPB = 10%) and the dry summer scenario (Scn_{DS}; ξ= 59%, SSPB = 45%), with approximately 80% within the range of a factor of 3 (Figure 5.5).

Based on the successful model evaluation of PECs, simulations for the entire Vecht River catchment were performed. In the Scn_{AC}, metformin, metoprolol, and carbamazepine had the highest PECs at watercourses affected by upstream STPs, with median concentrations

of 0.19 (0.01–3.03), 0.07 (2×10^{-3}–1.44), and 0.043 (2×10^{-3}–0.84) µg/L, respectively. Similarly, the highest median PECs in the Scn_{DS} were 0.57 (0.01–19.43), 0.25 (4×10^{-3}–4.08), and 0.18 (0.01–2.36) µg/L for metformin, metoprolol, and carbamazepine, respectively. The preceding median, minimum, and maximum PEC values exclude river segments with a PEC of zero. In previous studies, these APIs have been predicted or measured at similar concentration ranges in Dutch (Moermond et al., 2020; Oosterhuis et al., 2013) and German (Dusi et al., 2019; Meyer et al., 2016; Scheurer et al., 2009) surface waters. Although metformin is effectively transformed into guanylurea during wastewater treatment (Oosterhuis et al., 2013), it exhibited the highest PEC among the investigated APIs. This is a consequence of the high consumption of metformin (twelfth highest defined daily dosage [DDD] and seventeenth most frequently used in the Netherlands (Dutch National Health Care Institute, 2020)) and its relatively high excretion rate. The lowest PECs in watercourses affected by STP effluents were exhibited by 17α-ethinylestradiol and cyclophosphamide, with median concentrations in Scn_{AC} of 0.02 (3×10^{-4}–0.82) and 0.37 (0.01–9.64) ng/L, respectively. As for Scn_{DS}, the concentrations for 17α-ethinylestradiol and cyclophosphamide were estimated at 0.05 (2×10^{-4}–0.99) and 1.17 (2×10^{-4}–756.98) ng/L, respectively. These results were in line with the low consumption volumes of these APIs, despite a considerable fraction being excreted.

Concentration profiles of the Vecht River main stream are displayed in Figure 5.6 for the 8 APIs in the 2 exposure scenarios. The factors that cause differences in the PEC profiles observed along the main stream can be manifold and API-dependent. Erythromycin's low PECs in the Dutch regions coincide with the Dutch population's lower consumption patterns compared with their German counterparts. Persistent substances which are equally consumed on both sites of the border, such as carbamazepine, show higher PECs in Dutch regions because of the higher population density. Dilution ratios of treated effluent after entering the river system are lower if more people are connected to rivers with comparable flow rates.

The effect of dilution is also clearly visible in the PEC profiles of the 2 scenarios: dilution in Scn_{DS} is approximately 10 times lower than in Scn_{AC}. Lower flow rates lead to higher residence times and lower water levels in the river system, resulting in a larger influence of dissipation processes in Scn_{DS} than in Scn_{AC}. As a result, predicted summer concentrations of most APIs (17α-ethinylestradiol, carbamazepine, cyclophosphamide, erythromycin, metformin, and metoprolol) were on average a factor of 4 to 6 times higher than in Scn_{AC}. Among the APIs studied, ciprofloxacin was the compound most susceptible to dissipation processes, namely via direct photolysis, resulting in drastically lower PECs in Scn_{DS} than in Scn_{AC}. Diclofenac is also prone to direct photolysis. This in com-

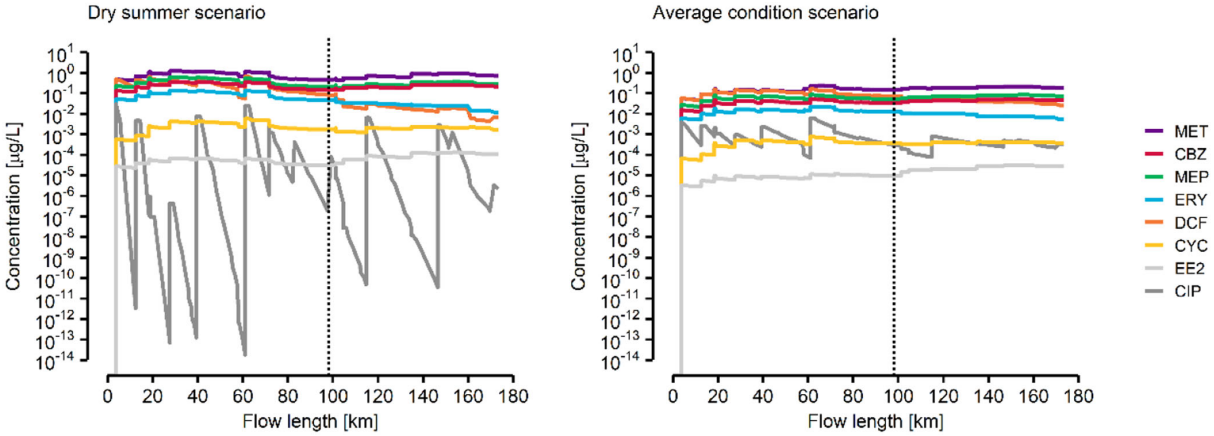

Figure 5.6: Predicted environmental concentrations of pharmaceuticals in the Vecht River main stream. The vertical black dashed line indicates the Dutch-German border. MET = metformin; CBZ = carbamazepine; MEP = metoprolol; ERY = erythromycin; DCF = diclofenac; CYC = cyclophosphamide; EE2 = 17α-ethinylestradiol; CIP = ciprofloxacin.

bination with lower consumption rates in the Netherlands helps explain the low PECs downstream of the border in the Scn_{DS} compared with Scn_{AC}.

5.3.2. PNECs

In the environmental effect assessment, there was a clear disparity in data availability for different substances. The lowest chronic PNEC was exhibited by 17α-ethinylestradiol (3.6×10^{-6} µg/L) and metformin the highest (440 µg/L). We revised existing chronic PNECs of the 8 APIs, including for diclofenac (0.01 µg/L), carbamazepine (0.02 µg/L), and cyclophosphamide (125 µg/L; Figure 5.7; Table B.15), which were 2, 2.5, and 4.5 times lower than the lowest PNECs reported previously in the literature or regulatory documents (Table B.16).

These lower PNECs give cause for concern regarding the environmental impact of these APIs and indicate the need to revise proposed EQSs for these APIs. For metoprolol and ciprofloxacin, the PNECs estimated in the present study were 310 and 78 µg/L, which are 5 and 156 times the highest PNECs found in the literature, respectively. It should be stressed that any PNEC can be strongly affected by the accessibility of effect data, the thoroughness of the search, and the quality assessment procedure (Henning-de Jong et al., 2009; Oelkers, 2020). This is illustrated by a suggestion we received from one of the anonymous reviewers, that is, to include the study of Ebert et al. (2011) in the derivation of the PNEC for ciprofloxacin. This is a critical study underlying the low ciprofloxacin PNEC of 0.089 µg/L listed in Table B.16, yet it was not retrieved from any of the sources used in the present study. It explains the large difference in derived PNECs for ciprofloxacin

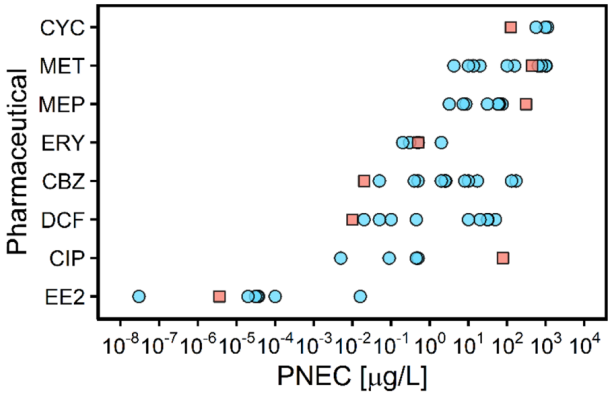

Figure 5.7: Predicted no-effect concentrations (PNECs) from the literature and derived in the present study. Salmon-colored squares indicate the PNEC values derived in the present study. Light blue points indicate unique PNEC values found in the literature. CYC = cyclophosphamide; MET = metformin; MEP = metoprolol; ERY = erythromycin; CBZ = carbamazepine; DCF = diclofenac; CIP = ciprofloxacin; EE2 = 17α-ethinylestradiol.

observable in Figure 5.7 and illustrates more generally that PNECs and risk assessment outcomes based on the assessment factor approach are very sensitive to the effect data included in the assessment. Indeed, the differences in PNECs for the same API derived by different agencies and assessors range from a factor of 10 to almost 106 (Figure 5.7). Keeping this range in mind, it is defendable to use an RQ of 0.1, or even smaller, as a potential indicator of risk and as a trigger to critically review and potentially improve the assessment procedure. To account for uncertainty in the derivation of PNEC values, an assessment factor of 50 was applied to diclofenac and 17α-ethinylestradiol, whereas an assessment factor of 10 was applied to carbamazepine, ciprofloxacin, cyclophosphamide, erythromycin, metformin, and metoprolol. The use of a relatively low assessment factor (instead of 100 or 1 000) suggests that the PNECs derived in the present study are not overly conservative.

5.3.3. Aquatic ecological risk

Single substance assessment

In the present study, RQ < 0.1 indicates a reason for no concern in terms of chemical pollution, $0.1 <$ RQ ≤ 10 indicates a potential reason for concern, and RQ > 10 suggests a reason for serious environmental concern. The specific boundary value(s) that qualifies as a "reason for concern" is malleable, depending on the empirical data that support it and personal values. In the present study, we chose to acknowledge the uncertainties that blur the meaning of this threshold (RQ $= 1$). Values of RQ > 1 can trigger follow-up measures, via either additional ecotoxicity testing or the implementation of risk management

measures (Posthuma et al., 2019; Zhou et al., 2019). In the present study, the PECs of 5 APIs were below their safe thresholds (PNECs). However, the PECs systematically exceeded PNECs in ascending order for diclofenac, carbamazepine, and 17α-ethinylestradiol (Figure 5.8). This observation holds for the average and dry summer scenarios, although risks were considerably higher in summer because of reduced dilution under dry weather conditions.

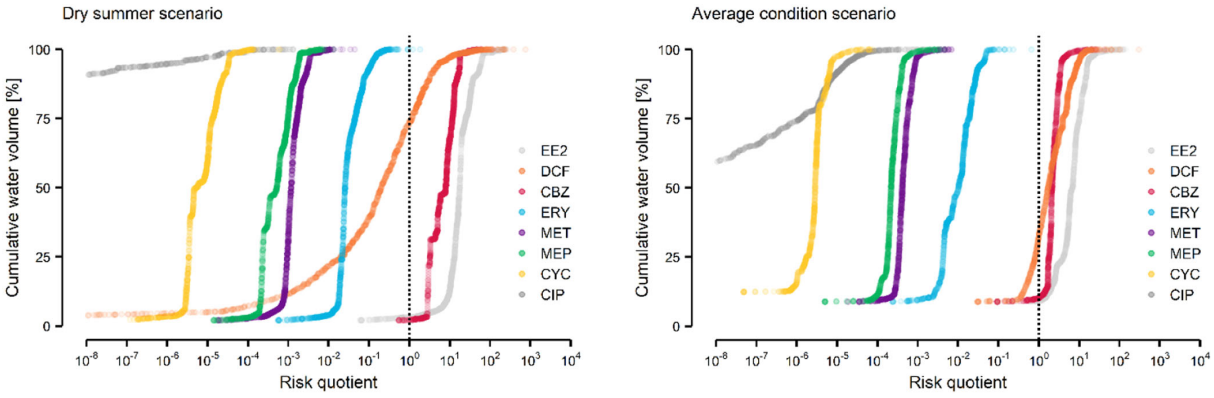

Figure 5.8: Percentage of the Vecht River catchment water volume at risk of environmental pharmaceutical pollution. Vertical black dashed line indicates the safe threshold, risk quotient = 1 (i.e. predicted environmental concentrations equal to the predicted no-chronic-effect concentration). In the average scenario, ciprofloxacin's risk quotients are $< 10^{-8}$; thus, they are not depicted. Each point depicts the relative water volume of a segment of ≤2 km. In the dry summer scenario, concentrations of ciprofloxacin $< 10^{-8}$ are also not depicted. EE2 = 17α-ethinylestradiol; DCF = diclofenac; CBZ = carbamazepine; ERY = erythromycin; MET = metformin; MEP = metoprolol; CYC = cyclophosphamide; CIP = ciprofloxacin.

Diclofenac, carbamazepine, and 17α-ethinylestradiol exceeded the safe PNEC threshold in at least 68 to 91% and 26 to 98% of the Vecht River catchment surface water volume during average conditions and dry summer conditions, respectively. In terms of the total flow length of all water bodies, the same APIs exceeded their PNECs in 31 to 38% and 24 to 53% during average conditions and dry summer conditions, respectively (Figure B.4). In the average condition scenario, ciprofloxacin, cyclophosphamide, erythromycin, metformin, and metoprolol do not pose a concerning risk to the aquatic life (i.e. 93 to 100% of the water volume had RQ ≤ 0.1). In the dry summer scenario erythromycin showed concerning risk levels (RQ > 0.1) in 17% of the catchment's water volume. 17α-ethinylestradiol exhibits the highest RQs despite showing the lowest PECs overall, with 25 and 87% of the catchment water volume showing concerning risk levels (RQ > 10) in the average and summer scenarios, respectively (Table B.17). In the Dutch municipality of Hengelo, 17α-ethinylestradiol showed a local risk of serious concern under average conditions in a small brook ($RQ_{\mathrm{ScnAC}} = 144$), whereas under dry summer conditions the risks were highest at local canals (< 2 km) routing STP effluents into larger streams and canals,

for example, Bornse Beek ($RQ_{\mathrm{ScnDS}} \leq 274$). This synthetic hormone has been shown to particularly interfere with the endocrine system of fish and amphibian species, affecting their development, reproduction, growth, and, ultimately, ability to sustain a healthy population (Table B.15). Eight of the 10 most sensitive species to ethinylestradiol identified in the present study are fish. Notably, Gobiocypris rarus (commonly known as rare minnow), a fish species endemic to China, is the most sensitive species (Zha et al., 2008). However, Rutilus rutilus (commonly known as roach) is a fish native to most European freshwaters including the Vecht River and is similarly sensitive (Lange et al., 2009). One study assessed the effect of wastewater estrogen exposure on roach population density in 2 English rivers over the span of a decade, finding no noticeable declines (Johnson and Chen, 2017). Another study analyzed the results of fish samples over a period of 2 decades in German rivers and found a decrease in fish population density, although it could not attribute it to chemical pollution (Teubner et al., 2019). To our knowledge, there are currently no indications that the roach is subject to adverse effects in the Vecht River basin. Nonetheless, the results of the present study support the use of more sensitive analytical techniques combined with accurately modeled hotspots of estrogen pollution and fish species in the Vecht River basin, including the roach. Furthermore, considering that the majority of the catchment was predicted to be liable to serious environmental risk, chronic effects could be triggered because continuous exceedance of an RQ of 1 is very likely under the simulated scenarios. At catchment locations, these exceedances can vary substantially, which can provide an opportunity for motile organisms to avoid unfavorable conditions or endure them for shorter exposure periods.

Carbamazepine exhibited the second highest RQs, with 90% of the catchment water volume showing concerning risk levels ($RQ_{\mathrm{ScnAC}} > 0.1$; Table B.17). Throughout the catchment, carbamazepine showed its highest risk ($RQ_{\mathrm{ScnDS}} = 118$, $RQ_{\mathrm{ScnAC}} = 42$) in a 7-km tributary segment under high-effluent influence, located in the German municipality of Bad Bentheim. Carbamazepine causes a variety of toxicological effects at different taxonomic levels. The most sensitive species include the insect Stenomena sp. (Jarvis et al., 2014), the crustacean Daphnia similis (Chen et al., 2019), the algae Chaetophora sp. (Jarvis et al., 2014), and the fish Pimephales promelas (Thomas et al., 2012), for which carbamazepine affects behavior, reproduction ability, or population survival. It is unclear whether these species are present in the Vecht River, but given carbamazepine's diverse ecotoxicological potential, targeted monitoring of its concentration levels and the sensitive Stenomena sp. could help determine whether adverse effects occur under field conditions.

Diclofenac exhibited the third highest RQs, with 90% of the catchment water showing concerning risk levels ($RQ_{\mathrm{ScnAC}} > 0.1$; Table B.17). At the same location in the German

municipality of Bad Bentheim, diclofenac showed the highest risk quotient (RQ_{ScnDS} = 754, RQ_{ScnAC} = 302). Provided the high risk at this and other locations along the Vecht River basin, toxicological effects on growth and development could be expected on fish and algae. The most sensitive species to diclofenac is the widespread invasive bivalve Dreissena polymorpha, which may be indicative of the vulnerability of this taxonomic rank (mollusks) and the trophic level it represents (primary consumers). These freshwater mollusks provide essential ecosystem services, are key elements of the food chain, and play a major role in removing contaminants from high volumes of water. At the regional and local scales, pharmaceutical pollution could exacerbate the impact on what is already the most threatened animal group in Europe (Cuttelod et al., 2011).

In a Dutch governmental report, carbamazepine and diclofenac have previously been identified as contaminants of environmental concern to aquatic organism in the Netherlands (Moermond et al., 2016); and, in a revised iteration, 17α-ethinylestradiol has also been identified as such, whereas carbamazepine was no longer of concern (Moermond et al., 2020). The revised PNECs in the present study suggest that the RQs of diclofenac and carbamazepine may be higher than anticipated (underestimated RQ). Exceptionally, erythromycin was also marginally predicted to occur at concentrations above the PNEC in the Vecht River catchment freshwater in a typical summer season (RQ = 1.8). In the river's main stream, RQs were low (RQ < 0.1), particularly in Dutch territory because of water dilution and lower consumption. Furthermore, erythromycin's degradation in the water column is not expected to be substantial because of the limited residence time of APIs in the Vecht River main stream of 4 to 12 d for average and low-flow conditions, respectively (Li and Cui, 2020; Liu et al., 2019a). However, the unaccounted veterinary use of erythromycin in the present study could elevate the risks.

Metformin does not stand out from our risk profiling. However, metformin's main metabolite, guanylurea, is found in surface waters in quantities of up to 50% of the administered parent compound (Oosterhuis et al., 2013). Because guanylurea has a lower PNEC (0.16 µg/L) than metformin itself (Caldwell et al., 2019), risk assessment of metformin should include the metabolite because it could pose a risk related to widespread metformin application. The need to consider transformation products in aquatic risk assessment has been stated by other authors (Celiz et al., 2009; Han and Lee, 2017).

Overall, 17α-ethinylestradiol, carbamazepine, and diclofenac may pose unacceptable environmental risks in at least 31% of the Vecht catchment flow length for average conditions. This risk aggravates up to 53% during summer, affecting 1 483 out of 2 772 km of total flow length (Figure B.4). The average RQ increased consistently across APIs by approximately 10-fold between the average and dry summer scenarios. However, the most striking changes in PEC were observed at the confluence of polluted streams, effluent- dominated

waters, or segments receiving STP effluents, with a few instances in which treated effluent discharge contributed up to 90% of the stream's volume. Other studies have also observed that proximity to STPs can more heavily influence pharmaceutical PEC than seasonality (Balaam et al., 2010; Musolff et al., 2009; Vieno and Sillanpää, 2014). Because of human activity near the river source, API emissions result in residue concentrations exceeding the PNEC as early as 20 km downstream the Vecht River. In agreement with the present study, diclofenac and carbamazepine have also been predicted to display a high environmental risk in other European and international rivers (Chaves et al., 2020; Palma et al., 2020). The APIs with the highest RQs in the present study (17α-ethinylestradiol, carbamazepine, diclofenac, erythromycin) have recently been removed from the Water Framework Directive watch list, which may lead to losing sight of their ecological impact despite their potential risk. This is also emphasized by Burns et al. (2018), who identify these substances as common top-priority APIs. In addition, a review on the development in the field of substances of emerging concern over the previous 20 yr emphasizes the exceedance of EQSs and the need for spatially explicit risk modeling approaches (Tiedeken et al., 2017). This review further supports the usefulness of generating spatially explicit risk profiles as conducted in the present study. Similar efforts open up the possibility for stakeholders to comply with the Water Framework Directive, starting with prioritizing APIs so that more refined and locally relevant targeted risk-management measures can be applied successfully.

Substance mixture assessment

In the Vecht catchment, a noticeable difference between the risk index in the average scenario and the dry summer scenario was observed (Figures B.5 and B.6). In the dry summer scenario, the mean risk index was estimated to be 3.4 times higher than in the average condition scenario. Likewise, the maximum risk indices were found in river segments of the Dutch municipalities of Hengelo and Coevorden under average and dry summer condition scenarios, respectively. This suggests that periods of dry, warm weather conditions in the Vecht River catchment may lead to risks to freshwater wildlife communities above the risks estimated for average weather conditions.

In the Vecht River main stream (Figure 5.9), the predicted cumulative risk in the polluted segments (i.e. risk index > 0) ranges between 6 to 22 and 23 to 104 in the average scenario and dry summer scenario, respectively. These risk index values in the main stream are lower than observed elsewhere in the catchment (Figures B.5 and B.6). However, this emphasizes the sustained cumulative risk in the Vecht River's main stream, particularly

driven by diclofenac in the German region and 17α-ethinylestradiol in the Dutch region (Figure 5.9).

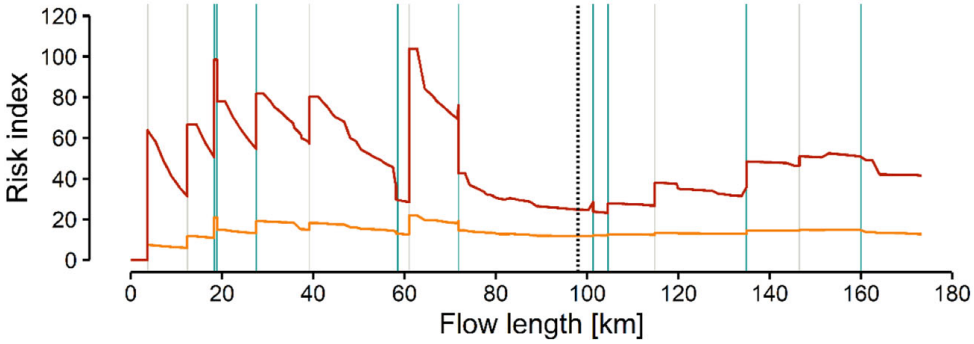

Figure 5.9: Risk index along the Vecht River main stream under typical dry summer (orange) and average weather (red) conditions. Eight pharmaceutical active ingredients are integrated in the risk indices depicted. Dashed vertical line demarks the German-Dutch border. Solid vertical lines depict sewage treatment plants (gray) and tributary confluences (turquoise).

5.3.4. Limitations

The present study embodies the ongoing attempt to predict API concentrations in freshwater and the associated risk of biological functional disturbance in regional ecosystems. Despite the advancements achieved, data scarcity, knowledge gaps, and procedural limitations often hamper the accuracy and significance of exposure and effect assessments. The sources of variability and uncertainty that can affect PECs and PNECs are manifold. The PEC can be affected by the excretion rate, sampling method, analytical chemistry technique, unaccounted point and diffuse emission sources, in-sewer (bio-) transformation, disposal of unused medicine in the toilet, or household wastewater (van Nuijs et al., 2015). For example, there are uncertainties linked to the German consumption rate of erythromycin, which seems to have been overestimated. Furthermore, erythromycin and ciprofloxacin PECs are associated with higher uncertainties because these were not sufficiently detected in the Vecht water system to allow for a corroboration with measurements. Similarly, the accuracy of model predictions for cyclophosphamide and 17α-ethinylestradiol could not be firmly determined because of analytical limitations. Indeed, concentrations of these APIs in surface water were often below their limits of detection and quantification. This is particularly important for assessing the risks associated with substances like 17α-ethinylestradiol because of its very low safe PNEC. Therefore, under such analytical limitations, the crucial contribution of predictive models is self-evident. The sensitivity of derived PNECs to data availability (e.g. effect studies that are missed, differently quality-assessed, or newly performed) is a typical feature of the assessment

factor method. The alternative SSD method is less affected by this phenomenon because it uses the 5^{th} percentile of the cumulative distribution function. As such, the sensitivity of PNECs to data availability also partly relates to the strict criteria on data availability that the European Union set for applying SSDs.

5.4. Conclusion

The present study achieved 3 main goals: 1) estimation of API surface water concentrations using the GREAT-ER model in the Vecht catchment; 2) derivation of new safe ecological threshold concentrations for 8 APIs, of which 3 were the lower than found in the literature; and 3) the creation of detailed, spatially explicit ecological risk profiles of APIs in a transboundary (sub-)catchment under 2 different seasonal scenarios. The exceedance of the acceptable ecological risk threshold in the Vecht River was found to be mainly driven by 17α-ethinylestradiol, diclofenac, and carbamazepine. These substances are among the most consumed APIs in the Netherlands. 17α-ethinylestradiol predominantly contributed to the aggregated risk profile and systematically exceeded the PNEC by at least one order of magnitude. This substance is the API with the twenty-third highest DDD and has seen a 4% increase from 2018 to 2019 (Dutch National Health Care Institute, 2020). This prospect emphasizes the need for better pharmaceutical emission reduction strategies (e.g. wastewater treatment technology, hotspot analysis, and preventive health care) and continue to monitor its use and presence in surface waters (Government of the Netherlands, 2019), including the Vecht River. The present study suggests that the Vecht River catchment is vulnerable to pharmaceutical pollution, with 26 to 98% of its surface waters and 24 to 53% of its length under potentially unacceptable ecological risk (RQ $>$ 1), particularly during a dry summer season. European regulation demands that national and regional authorities take action in securing water bodies' good status. To this end, the present study demonstrated the value of tailor-made regional models and the continuous revision of ecotoxicological information. Furthermore, it highlighted the importance of assessing off-site risks of pharmaceutical emissions using (sub-)catchment modeling across national borders, therefore emphasizing the imperative for international cooperation. Ultimately, these results should encourage further cross-boundary action and initiative from local authorities to comply with environmental standards via feasible and locally relevant risk-management strategies. Otherwise, risk reduction implementations in international river networks may not be sufficiently effective.

5.5. Acknowledgments

We thank the regional hospitals for providing pharmaceutical consumption information and D. de Zwart for kindly sharing e-toxBase data. The present study was supported by the European Regional Development Fund of the European Union under the project MEDUWA Vecht(e) (142118).

Original publication: Environmental Science and Technology **56**(21): 15064–15073 (2022)
available at http://dx.doi.org/10.1021/acs.est.2c00218

(Antibiotic-resistant) *E. coli* in the Dutch-German Vecht catchment - monitoring and modeling

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Abstract

Fecally contaminated waters can be a source for human infections. We investigated the occurrence of fecal indicator bacteria (*E. coli*) and antibiotic-resistant *E. coli*, namely ESBL-producing *E. coli* (ESBL-EC) and carbapenemase-producing *E. coli* (CP-EC) in the Dutch-German transboundary catchment of the Vecht River. Over the course of one year, bacterial concentrations were monitored in wastewater treatment plant (WWTP) influents and effluents and in surface waters with and without WWTP influence. Subsequently, the GREAT-ER model was adopted for the prediction of (antibiotic-resistant) *E. coli* concentrations. The model was parameterized and evaluated for two distinct scenarios (average flow scenario, dry summer scenario). Statistical analysis of WWTP monitoring data revealed a significantly higher (factor 2) proportion of ESBL-EC among *E. coli* in German compared to Dutch WWTPs. CP-EC were present in 43% of influent samples. The modeling approach yielded spatially accurate descriptions of microbial concentrations for the average flow scenario. Predicted *E. coli* concentrations exceed the threshold value of the Bathing Water Directive for a good bathing water quality at less than 10% of potential swimming sites in both scenarios. During a single swimming event up to 61 CFU of ESBL-EC and less than 1 CFU of CP-EC could be taken up by ingestion.

6.1. Introduction

The application of antibiotics to treat previously incurable diseases has improved both, life quality and expectancy. However, with the increasing use of antibiotics, the relative number of diseases caused by antibiotic-resistant pathogens has also increased in the last decades (van Duin and Paterson, 2016). The emission of fecal bacteria into the environment from human excrements occurs via discharge of (treated) wastewater (Heuer et al., 2011; Rizzo et al., 2013). This also encompasses many antibiotic-resistant species. Besides these point sources, diffuse sources comprise of (i) direct runoff from areas with fecal contamination, e.g. from manure fertilization (Meals and Braun, 2006), (ii) direct drop-off of feces by pasture animals with direct water access (Muirhead, 2015), (iii) excretions of wildlife (Parajuli et al., 2009), especially water fowl (Ewers et al., 2009; Hansen et al., 2020), and (iv) remobilization of particle bound and trapped bacteria, from the sediments (Grant et al., 2011).

Fecal contamination of surface water is usually assessed via indicator bacteria like the intestinal bacteria Escherichia coli (*E. coli*), as laid down in the European Bathing Water Directive (European Union, 2006). In the last years, antibiotic-resistant bacteria such as extended spectrum beta lactamase producing *E. coli* (ESBL-EC) and carbapenemase-producing *E. coli* (CP-EC) gained large interest. ESBL-EC are resistant towards third and fourth generation beta-lactam antibiotics but not against carbapenem antibiotics. They have been frequently found in effluents of municipal wastewater treatment plants (WWTPs) (Blaak et al., 2015a; Korzeniewska et al., 2013), manure (Friese et al., 2013; Schmitt et al., 2019), surrounding areas of livestock buildings (Blaak et al., 2015b; Gao et al., 2015), surface waters (Blaak et al., 2014) and sediments (Amos et al., 2015). CP-EC are of particular concern, as they are also resistant towards carbapenems, i.e. last resort antibiotics (Grundmann et al., 2017). In a nationwide study in the Netherlands, CP Enterobacterales (CPE) have been detected in 89 of 100 monitored WWTPs (Blaak et al., 2021).

Considerable efforts have already been undertaken to monitor *E. coli* in surface water and wastewater (e.g. Blaak et al., 2014, 2021). While these studies focus mainly on specific river sites downstream of known emission sources, some more comprehensive catchment wide monitoring campaigns have been performed to quantify the impact of different emission sources, get insight in their environmental fate and assess the status of fecal contamination (e.g. McKergow and Davies-Colley, 2009; Nakhle et al., 2021; Ouattara et al., 2011). Catchment wide studies on ARB, however, have rarely ever been carried out. Serwecińska et al. (2021) were the first to provide an overview of the occurrence of carbapenem-resistant Acinetobacter spp. on catchment scale. For ESBL-EC and CP-EC, such comprehensive studies on the level of entire catchments do not exist.

Reaching conclusions beyond those from monitoring, fate modeling of bacteria in whole catchments can help understanding underlying processes, comparing the importance of different emission sources and identifying contamination hotspots. Especially when concentrations are close to or below the detection limit, e.g. with CP-EC given their rather low concentrations in WWTP effluent (Blaak et al., 2021), predictive models can be useful tools to complement monitoring. The GREAT-ER (geography-referenced regional exposure assessment tool for European rivers) model is well established for simulating chemical exposure in whole river catchments. It has been successfully applied to predict environmental concentrations of different chemicals like detergents (Schowanek et al., 2001), pharmaceuticals (Alder et al., 2010; Kehrein et al., 2015; Lämmchen et al., 2021c) and even dissolved zinc (Hüffmeyer et al., 2009) in various catchments. Recently, it has been applied for risk assessment of selected pharmaceuticals in the Dutch-German transboundary catchment of the Vecht River (Duarte et al., 2021). The model covers

processes as emissions from WWTPs as point sources, in-stream transport, sedimentation and degradation, in a steady-state approach. While temperature and precipitation resolved monitoring is needed to identify risks during particular conditions (e.g. during overflows after heavy rains which can increase concentrations greatly), steady-state models can be used to map gradients in average risks. In turn, this helps identifying locations of lower and higher concern, which could be studied in more detail if needed.

The current study has two objectives. The first aim is to get insight into the spatio-temporal distribution and dynamics of (AR) *E. coli* in surface waters and wastewaters by combining a comprehensive one-year monitoring campaign in the catchment of the Dutch-German cross-border Vecht River with catchment modeling. We analyzed *E. coli*, ESBL-EC and CP-EC in surface waters with and without WWTP influence as well as in WWTP influents and effluents. Then, the GREAT-ER model was extended to include a simulation routine for simulating (AR) *E. coli*. Predicted concentrations are evaluated against measured data. The second aim is to perform an exposure assessment during average weather conditions on catchment scale based on predicted microbial concentrations.

6.2. Materials and Methods

6.2.1. Study area

The catchment area of the Dutch-German transboundary Vecht River, a tributary of the Dutch IJssel River, extends over an area of about 6 100 km^2 (Figure 6.1). Emissions from approximately 1.5 million inhabitants connected to 57 WWTPs enter the Vecht River or its tributaries. In addition, the wastewater of 13 hospitals is treated by these WWTPs. The catchment is characterized by high proportions of agricultural land use (Table C.1). Further details are described in Duarte et al. (2021) and Wöhler et al. (2020).

6.2.2. Monitoring campaign

The monitoring campaign was run from July 2018 to August 2019 and included samples from 41 different locations (10 WWTPs and 31 surface water sites) spread over the entire Vecht catchment (Figure 6.1). Each location was sampled monthly with a maximum of 10 sampling moments. Exact coordinates of all sampling locations and sampling site IDs can be found in Table C.2. All samples were analyzed for *E. coli* and ESBL-EC. CP-EC were only cultured from influent and effluent samples as in-stream concentrations were expected to be below the limit of quantification (LOQ). Sampling procedure and quantification methods can be found in Appendix C (Texts 1 and 2). In short, concentrations

of *E. coli*, ESBL-EC and CP-EC were determined by enumeration based on selective agar plates. TBX agar was used for isolation of *E. coli*, Chromagar ESBL for ESBL-EC, and ChromID CARBA for non-OXA-48 CPE (Blaak et al., 2021). Membrane filtration of a range of water volumes was used to generate bacterial concentrations (ISO, 2018). 5–10 isolates from each sample were species confirmed by indole testing, and for a selection of isolates, phenotypic ESBL resistance and species identity was confirmed by VITEK-MS (BioMérieux, Amersfoort, the Netherlands).

Ten WWTPs were selected for monitoring based on the plant location (Germany: 4, Netherlands: 6), scale (9 000–180 000 inhabitants), and whether the plant treated hospital wastewater (5) or not (see Table C.3). WWTP influent and effluent samples were collected at the same time. All WWTPs use conventional conventional activated sludge (CAS) treatment; two WWTPs have a hybrid treatment system combining CAS treatment with advanced treatment techniques.

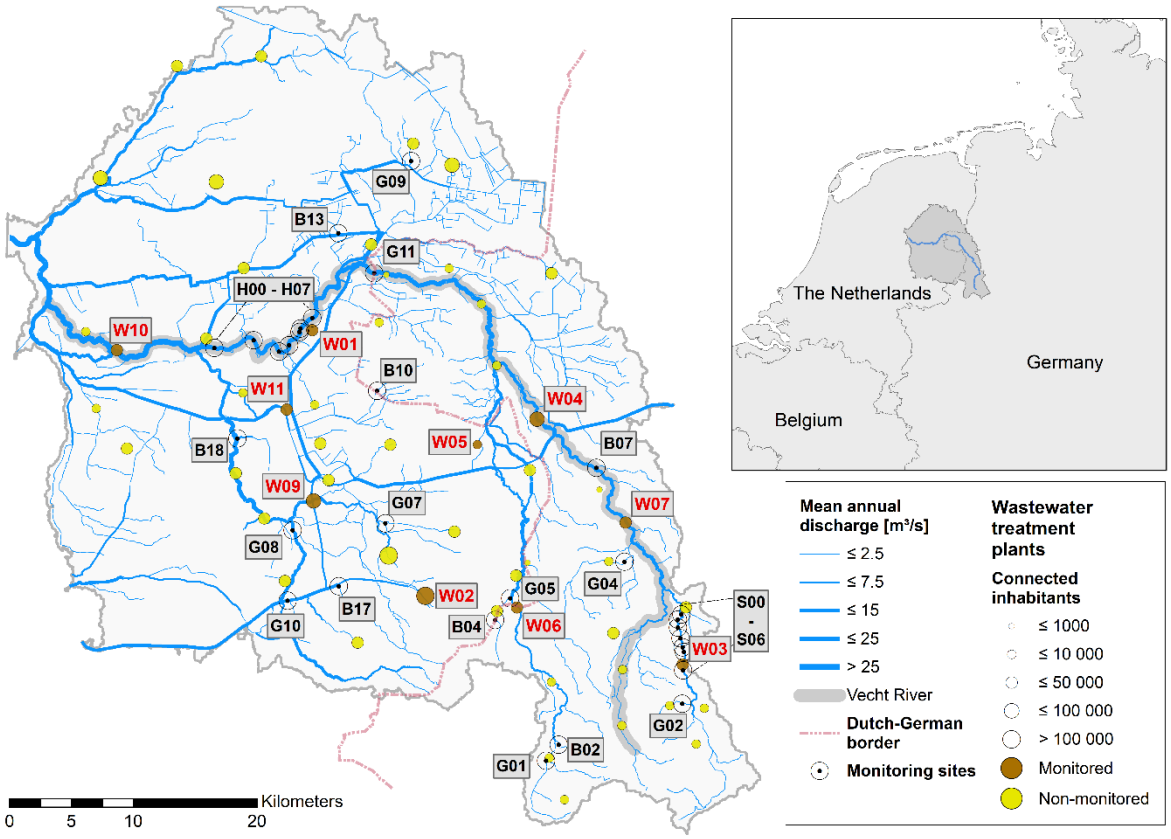

Figure 6.1: Overview map of the Vecht catchment area, monitoring sites and monitored wastewater treatment plants (WWTPs). Letters in monitoring site IDs indicate different sampling site types: W: WWTP samples, B: background samples in areas without WWTP influence, S and H: longitudinal concentration profiles at WWTPs W03 and W01, respectively, G: general catchment samples.

Surface water sampling sites were selected to deliver data for three different situations, namely, longitudinal concentration profiles downstream of WWTPs W01 (NL) and W03 (GE), background locations without known WWTP effluent emissions (background samples B), and evaluation data from sites across the whole catchment with at least one WWTP emission upstream (general catchment samples G). Longitudinal profile samples included one reference site located upstream of the WWTPs W01 (H00) and W03 (S00) and several downstream sites over distances of 16.7 km (H01–H07) and 9.0 km (S01–S06), respectively (Table C.2). No other point emissions are known to occur within these distances. The data were used to investigate (i) the local influence of WWTP emissions on *E. coli* concentrations and (ii) temporal variation along a longitudinal gradient. Background sites were selected as representative of diffuse bacterial sources with a focus on the effect of agricultural land use determined by the help of land use maps (cropland and pasture, Table C.4). General catchment locations (G02–G11) are affected by at least one WWTP emission. Criteria for the selection of sampling sites were their proximity to gauging sites and their spatial distribution in the catchment to provide a useful basis for evaluation of the GREAT-ER model simulations.

In total, 196 WWTP influent and effluent samples, 70 background samples, 72 and 62 samples for the longitudinal profiles W01 and W03, respectively, and 90 general catchment samples were collected and analyzed (Tables C.5–C.6).

6.2.3. Evaluation of wastewater treatment plant samples

Assuming constant *E. coli* loads per person, measured influent and effluent concentrations are transformed into per capita loads ($pcL_{B,m}$ [CFU cap^{-1} d^{-1}]), similarly to Pallares-Vega et al. (2021). Data on the number of inhabitants connected to the WWTPs as well as discharges at the sampling days were provided by the respective authorities and water boards (Table C.7).

$$pcL_{B,m} = \frac{C_{B,m} \times Q}{Inh} \times 1000 \tag{6.1}$$

$C_{B,m}$ [CFU L^{-1}] is the concentration of bacteria B (*E. coli*, ESBL-EC or CP-EC) in sampling matrix m (influent or effluent), Q [m^3 d^{-1}] is the WWTP discharge of the respective day, Inh [cap] is the number of connected inhabitants and 1 000 the unit conversion factor from m^3 to L. It was assumed that WWTP discharge was the same for influent and effluent at a given date.

WWTP treatment efficiency (bacterial reduction $logRed_B$) was calculated from the logarithms of influent ($C_{B,in}$ [CFU L^{-1}]) and effluent concentrations ($C_{B,eff}$ [CFU L^{-1}]) of the respective bacteria B (Schijven et al., 2015a):

$$logRed_B = -log_{10}\frac{C_{B,eff}}{C_{B,in}} \tag{6.2}$$

Since detection frequencies of CP-EC in effluents were too low, reduction was calculated for *E. coli* and ESBL-EC only.

Relative abundances of AR *E. coli* ($r_{ARB,m}$) in influent and effluent (matrices m) are calculated by normalizing their concentrations with total *E. coli* concentrations, following published approaches (Marano et al., 2020; Pilmis et al., 2021).

$$r_{ARB,m} = \frac{C_{ARB,m}}{C_{E.\ coli,m}} \tag{6.3}$$

We evaluated the contribution of different factors on *E. coli* influent loads, log reduction and relative abundance of AR *E. coli* (ESBL-EC and CP-EC) to total *E. coli* using linear mixed models, with WWTP as random factor to correct for clustering of observations within WWTPs. Response variables and explanatory variables are displayed in Table C.8. The analysis was performed in R (version 3.6.3) using packages lme4 and lmerTest (Bates et al., 2015; Kuznetsova et al., 2017). The final models were inspected for heteroscedasticity and homogeneity of variances.

6.2.4. Modeling microbial water quality in the Vecht catchment

The GREAT-ER model

The GREAT-ER model is a spatially resolved river catchment model following a mass balance approach and assuming steady-state conditions. The model was originally developed to predict aquatic exposure concentrations of down-the-drain chemicals in entire catchments (Feijtel et al., 1998; Lämmchen et al., 2021c). Contaminants are traversed as loads through the river network with a spatial resolution of 2 km flow length. Final concentrations are derived by dividing simulated loads with the discharge of the respective river segment. A conceptual representation of the model is provided in Appendix C (Figure C.1). The GREAT-ER software is implemented as an Add-In for the geographic information system ESRI ArcGIS Desktop® versions 10.0 and higher. Technical details are provided in Kehrein et al. (2015) and Lämmchen et al. (2021c).

Hydrological representation of the Vecht catchment

The Vecht catchment is characterized by strong anthropogenic influence on the hydrological conditions. Especially in the Netherlands, a network of canals has been installed to keep water levels in the Vecht River constant in summer for year-round navigability. Additionally, tributaries are prevented from falling dry by pumping IJssel water into the area. This makes the usual stochastic description of the natural flow rate variation virtually impossible. Instead, two different scenarios describing season-specific typical hydrologic conditions were created for the Vecht catchment. A detailed description of the set-up and the hydrological representation is provided by Lämmchen et al. (2021b). Its general applicability was recently confirmed by a case study with pharmaceuticals (Duarte et al., 2021; Lämmchen et al., 2021b). The first scenario represents the situation of dry weather in summer (dry summer scenario) and the second one describes humid periods throughout the whole year (average flow scenario), where the flow rate is affected by interflow and surface runoff due to precipitation (see Table C.9 for more details).

Emission estimation

Average daily excretion of *E. coli* per person is assumed constant. *E. coli* emissions from WWTPs are thus modelled analogous to pharmaceuticals assuming a constant per capita emission rate pcL_{in} [CFU cap^{-1} d^{-1}] coupled with the logarithmic reduction efficiency of bacterial loads by wastewater treatment ($logRed$ [-]):

$$L_{eff} = pcL_{in} \times Inh \times 10^{-logRed} \tag{6.4}$$

L_{eff} [CFU d^{-1}] is the daily effluent load and Inh [cap] is the number of inhabitants connected to the WWTP. The contribution of different processes to *E. coli* concentrations in rural areas has been investigated earlier, e.g. by applying the SWAT model (Kim et al., 2010; Parajuli et al., 2009; Park et al., 2017). However, due to a lack of quantitative information about relevant parameters (e.g. sediment concentrations, wildlife coverage, groundwater exchange, and runoff data across the whole catchment), diffuse emissions of *E. coli* are modelled by a simple empirical approach summarizing all contributions in one parameter, namely a constant concentration C_B [CFU L^{-1}] in background inflow. Local emission loads (I_B [CFU d^{-1}]) for each river section are generated by multiplying this concentration with the flow increment between two adjacent river segments ΔQ [m^3 s^{-1}]

$$I_B = \Delta Q \times C_B \times 1\,000 \times 86\,400 \tag{6.5}$$

1 000 and 86 400 are conversion factors from m^3 to L and from days to seconds.

Parameterization of WWTP and diffuse emissions was based on measured microbial concentrations from WWTP and background monitoring sites.

Fate of *E. coli* in surface water

Due to the small size, the settling velocity of free *E. coli* bacteria in rivers is very slow. Efficient settling only occurs when the bacteria are attached to suspended material (Pachepsky and Shelton, 2011). Sedimentation of *E. coli* thus depends on the fraction of bacteria attached to suspended particles (f_s) and the settling velocity (v_{set} [m h^{-1}]) of these particles. The settling velocity of 0.1 m h^{-1} is adopted from the *E. coli* study in the Scheldt catchment (Ouattara et al., 2013). Sedimentation is then parameterized as first order process with settling rate k_{set} [h^{-1}] and water depth d [m] (Jamieson et al., 2005a):

$$k_{set} = \frac{v_{set}}{d} \tag{6.6}$$

Specific information about the attachment behavior of *E. coli* in the Vecht catchment was not available. Therefore, we aggregated data on *E. coli* attachment to suspended particles observed in other catchments resulting in a median fraction of 36.5% (Table C.10). The same parameters were also used to simulate ESBL-EC and CP-EC concentrations.

Survival of *E. coli* in surface waters depends on several environmental factors including water temperature, solar radiation, pH, salinity, nutrient availability and predation (Jozić and Šolić, 2017; Petersen and Hubbart, 2020). Among them, water temperature is commonly regarded as the major factor (Blaustein et al., 2013). Temperature dependent inactivation (die-off) of free floating *E. coli* can be described by a first order rate constant k(ϑ) [h^{-1}] (Blaustein et al., 2013; Chick, 1908), which is temperature corrected according to Ouattara et al. (2013):

$$k(\vartheta) = k_{20} \times \frac{\exp\left(\frac{-(\vartheta-25)^2}{400}\right)}{\frac{-25}{400}} \tag{6.7}$$

ϑ [°C] is the temperature and k_{20} [h^{-1}] is the inactivation rate at 20 °C. The temperature correction has been successfully applied to explain the dynamics of fecal coliforms in the catchment of river Scheldt for k_{20} set to 4.5×10^{-2} h^{-1} (Ouattara et al., 2013). Temperatures for the average flow scenario (11.9 °C) and the dry summer scenario (18.2 °C) were extracted from daily measurements in the catchment in the years 2016–2019. Inactivation rates of ESBL-EC and CP-EC were assumed the same as for *E. coli*. Inactivation rates of bacteria associated to suspended particles are set to 50% of the rate for free floating bacteria (Garcia-Armisen and Servais, 2007).

Model training and performance evaluation

Data from WWTP samples and background samples were used for the parameterization of emissions. WWTP samples were evaluated with respect to seasonal and national differences to estimate average values for pcL_{in} and $logRed$ in the two scenarios (for more details see Appendix C, Text 3 with Tables C.11–C.13). Average *E. coli* background concentrations (C_B) were estimated by fitting observed concentrations at background sites to the model (for more details see Appendix C, Text 4 with Table C.14). For both, WWTP and diffuse emissions, AR bacteria emissions were estimated based on observed relative abundances. Sampling site G10 is used for model parameterization of loads entering the Vecht catchment from the IJssel via the Twente Canal.

Model performance was evaluated by comparing predicted with measured surface water concentrations from general catchment samples and longitudinal profile samples. For the latter, only the upstream sampling locations (S00 and H00) and the sampling locations farthest downstream (S06 and H07) were included to avoid bias from correlated data within longitudinal profiles. Summer measurements (i.e. June 21 to September 22) were allocated to the dry summer scenario and the remaining samples to the average flow scenario. Since measured bacterial concentrations range over several orders of magnitude, the evaluation was performed on log-transformed data. GREAT-ER simulations reflect steady state without temporal resolution and variation caused by differences in e.g. flow and temperature. Thus, monitoring data at individual sites were aggregated into median values for comparison, whereby analysis data below the LOQ were processed as the respective concentration.

The coefficient of determination (R^2) and the percentage bias (PBIAS) were used as model performance metrics for *E. coli* and ESBL-EC separately for each of the two scenarios. This was not possible for CP-EC because it was not analyzed in surface water samples (see Section 6.2.2). R^2 describes the proportion of the variance in the measured data which can be explained by the model and is widely used for the evaluation of water quality models (Moriasi et al., 2007). Statistical significance (p-value) of R^2 was examined by calculating the F-statistic. PBIAS indicates if predicted concentrations are rather overestimate (PBIAS > 0) or underestimate (PBIAS < 0) observed concentrations (Moriasi et al., 2007).

Human exposure assessment

Surface waters are being used for recreational purposes, not only at designated bathing sites that are regularly monitored (Meijs et al., 2020; Schowanek et al., 2001). The Vecht

catchment in particular is frequently used for recreational purposes including swimming (Bréchet et al., 2014). The *E. coli* threshold value for good bathing water quality in terms of fecal contamination is defined in the Bathing Water Directive as 10 000 CFU L^{-1} (European Union, 2006). For the exposure assessment, we assume waterbodies with an average depth of at least 0.5 m to be potential swimming sites and evaluate predicted *E. coli* concentrations against the threshold at these sites for both scenarios. Since no such threshold exists for AR *E. coli*, we estimate the amount taken up during a single swimming event based on a swallowed water volume of 18 and 27 mL for women and men, respectively (Locatelli et al., 2020).

6.3. Results and Discussion

6.3.1. Monitoring overview

Log-transformed *E. coli* concentrations in WWTP influents were 7.92 ± 0.35 log CFU L^{-1} and 5.21 ± 0.74 log CFU L^{-1} in effluents. ESBL-EC loads in influent and effluent wastewater were approximately 2–3 log units below respective *E. coli* concentrations, namely 5.97 ± 0.47 and 3.25 ± 0.71 log CFU L^{-1}, respectively (Figure C.2). All values are in the range as of previously reported data, (e.g. Blaak et al., 2021, 2015a; Reinthaler et al., 2003). *E. coli* concentrations in background surface water samples were about two orders of magniture lower than effluent concentrations (3.10 ± 0.85 log CFU L^{-1}), and general catchment samples were slightly higher contaminated (3.46 ± 0.81 log CFU L^{-1}). The detection frequency of ESBL-EC in surface water was 67% and 39% for general catchment and background sites, respectively. CP-EC were only detected in 43% of influent and 16% of effluent samples and concentrations were lower than for ESBL-EC. Summary statistics are provided in the Appendix C (Tables C.15–C.19).

6.3.2. Analysis of wastewater samples with linear mixed models

E. coli per capita influent loads amounted to $pcL_{\textit{E. coli}, \text{influent}} = (2.2 \pm 1.8) \times 10^{10}$ CFU cap^{-1} d^{-1}. According to linear mixed models on data from all WWTPs, the *E. coli* influent load was independent of WWTP discharge and country, but showed significantly higher values in summer ($p < 0.001$, Tables C.11– C.12).

Log *E. coli* reduction in the investigated WWTPs (2.68 ± 0.9) was in the upper region of reduction values reported for conventional wastewater treatment (between 1 and 3 log units) (Barrios-Hernández et al., 2020; Blaak et al., 2015a; Galvin et al., 2010;

Korzeniewska et al., 2013; Pallares-Vega et al., 2021; Reinthaler et al., 2003). No difference between *E. coli* and ESBL-EC removal was observed ($p >> 0.1$). Effluent samples showed larger variation in per capita loads, both for *E. coli* and ESBL-EC, but no apparent seasonal trend of effluent concentrations was recognizable (Figure C.3). Linear mixed modeling showed that log reduction was inversely correlated with normalized WWTP discharge as proxy for reinfall ($p < 0.001$), probably due to lower residence time in the WWTP at higher discharge (Pallares-Vega et al., 2021).

Relative abundance of ESBL-EC (ESBL-EC to *E. coli* ratio) was significantly higher in the German WWTPs (factor 2 on average) compared to the Netherlands ($p < 0.001$). To the best of our knowledge, this is the first study showing national differences in relative ESBL-EC abundance in wastewater, albeit on a regional scale. Higher ESBL-EC abundances may be attributed to differences in prevalence between countries. Approximately 5% of the Dutch population carries ESBL-EC (Blaak et al., 2021). In Germany, a prevalence of 6.8% has been reported in 2015 (Lübbert et al., 2015), and 2.3% in 2015–2017 (Ny et al., 2018) albeit both determined with a slightly less sensitive method (Lübbert et al., 2015; Ny et al., 2018). Comparing the Dutch-German border region, no difference between the countries had been found earlier for the ESBL prevalence at hospital admittance (Zhou et al., 2017). Our findings suggest a difference in community prevalence in the cross-border region of the Vecht catchment, which might have been left unnoticed in the population studies due to differences in methodology, or due to the focus on specific populations such as hospital patients.

Relative abundance of ESBL-EC was 0.14 log units (40%) higher in summer than in the remaining year ($p < 0.001$). Seasonal effects on ESBL-EC carriage have been found in population studies of ESBL population prevalence, possibly related to travel to non-European countries or environmental exposure (e.g. due to recreational activities) as risk factors for increased ESBL carriage in summer (Lübbert et al., 2015; Meijs et al., 2020).

Concentrations of CP-EC relative to *E. coli* in influent of positive samples were in the same order of magnitude (10^{-5}) as reported by Blaak et al. (2021). For relative CP-EC abundance, neither country nor season showed a significant effect.

6.3.3. Temporal variation in longitudinal concentration profiles

The local impact of WWTP emissions on downstream *E. coli* concentrations in receiving rivers has been documented in several studies, (e.g. Bréchet et al., 2014; Reinthaler et al., 2003). Our data corroborate the local impact of WWTP effluent on downstream *E. coli* concentrations (see Figure 6.2), but the effect does not consistently occur at all sites and time points. The W01 profiles (Figure 6.2a) show no measurable effect of WWTP emission

on *E. coli* and ESBL-EC concentrations at three of the ten time points (December 2018, and February–March 2019). In February and March, the Vecht River at W01 exhibited high flow rates (three times the long-term annual average) together with high upstream concentrations. The latter might have been caused by storm water runoff or combined sewage overflows (CSO), where *E. coli* concentrations have been reported to be up to two orders of magnitude higher than in the WWTP effluent in this study (Locatelli et al., 2020; Passerat et al., 2011). Contributions from diffuse runoff can also significantly increase in-stream concentrations of *E. coli* after rain events (Kistemann et al., 2002; McKergow and Davies-Colley, 2009). One process behind this can be remobilization of bacteria from the sediments by turbulent mixing at high flow rates (McKergow and Davies-Colley, 2009). In July, monitoring data showed inexplicable profile dynamics at the longitudinal profile W01.

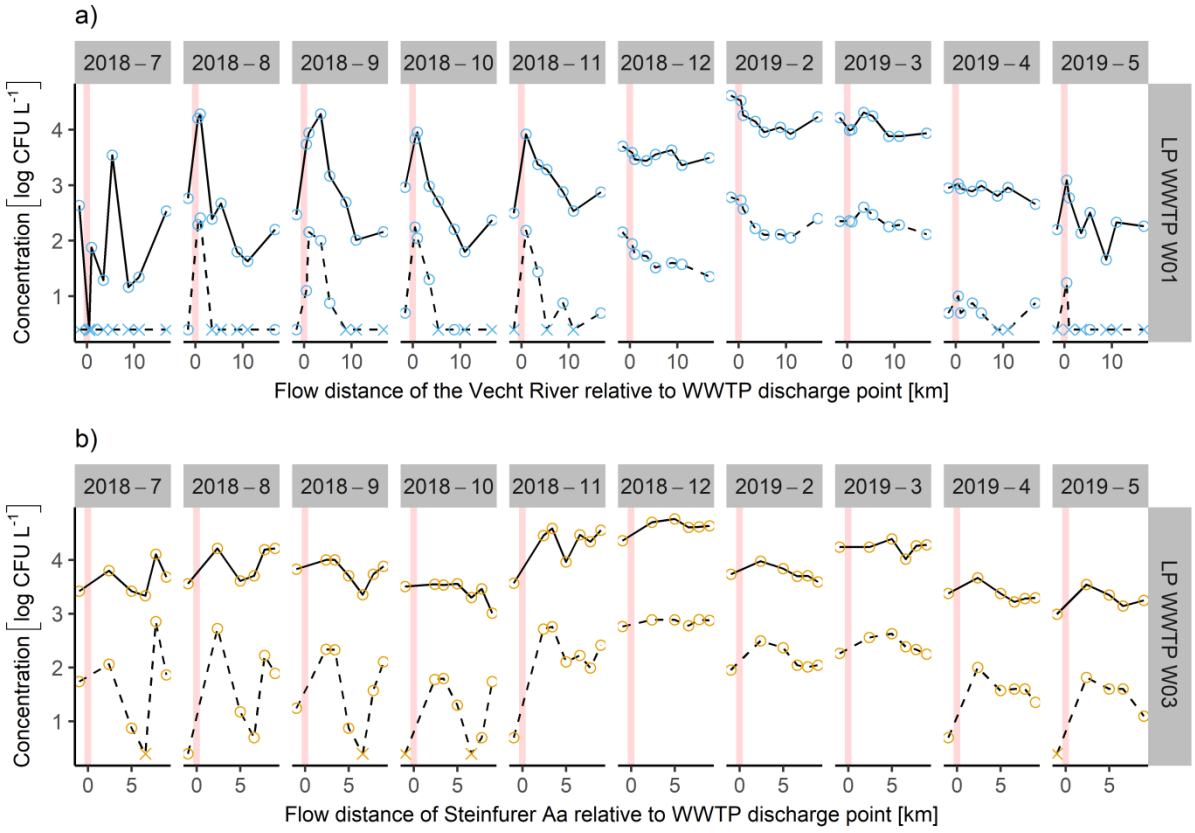

Figure 6.2: Measured concentrations of *E. coli* (solid lines) and ESBL *E. coli* (dashed lines) at longitudinal profiles (LPs) relative to discharge points of WWTPs W01 (a) and W03 (b). Crosses indicate concentrations below LOQ displayed as LOQ. Red lines indicate WWTP discharge points, i.e. relative flow distance of 0 km.

E. coli concentrations upstream of WWTP W03 (Figure 6.2b) are generally higher (on average by factor 3.6) than upstream of WWTP W01. Downstream of the WWTP, a concentration increase of 0.36 ± 0.28 log units is observed (Figure 6.2b). This effect is

even more pronounced for ESBL-EC with an increase of 1.08 ± 0.75 log units, most likely because the relative abundance of AR bacteria in the diffuse background is lower compared to WWTP effluents. This effect is particularly large in months July-November 2018 and April-May 2019. At the sampling point 6.6 km downstream of the WWTP, concentrations almost consistently increase again, which hints at an unknown point source. For the sampling months December 2018, February and March 2019, the WWTP emission effect is less clear.

6.3.4. Overall model evaluation

Simulated concentrations well explained the observed spatial variability of measured concentrations (represented by the spatial median) in the average flow scenario for *E. coli* ($p < 0.001$) and ESBL-EC ($p < 0.01$) (Figure 6.3). In the dry summer scenario, prediction accuracy was weaker (less significant R^2) for both, *E. coli* and ESBL-EC. Interestingly, simulations applying individual on-site monitoring data for WWTP emissions did not increase the prediction accuracy compared to the assumption of average per capita loads.

However, simulations showed a tendency towards overestimation (PBIAS > 0); e.g. at the longitudinal profile of WWTP W01, concentrations were overestimated. Overestimations were higher for ESBL-EC. ESBL-EC emissions are based on observed ESBL-EC abundances in WWTPs and at background sites relative to *E. coli* (Appendix C, Texts 3 and 4). ESBL-EC abundances below LOQ were not considered for parameterization of background sites, which may have introduced a bias towards higher input assumptions. Different in-stream inactivation and sedimentation rates for *E. coli* and ESBL-EC are unlikely, since ESBL-gene carriage has not been found to influence the fitness of *E. coli* (Ranjan et al., 2018; Schaufler et al., 2016).

We conclude that application of generalized parameters to predict *E. coli* and ESBL-EC concentrations is feasible for aquatic exposure assessment in the Vecht catchment. Especially in the average flow scenario, the model correctly depicts spatial variations. Under dry weather conditions, better understanding of the contribution of diffuse emission processes and respective model refinement are necessary to increase the accuracy of the predictions.

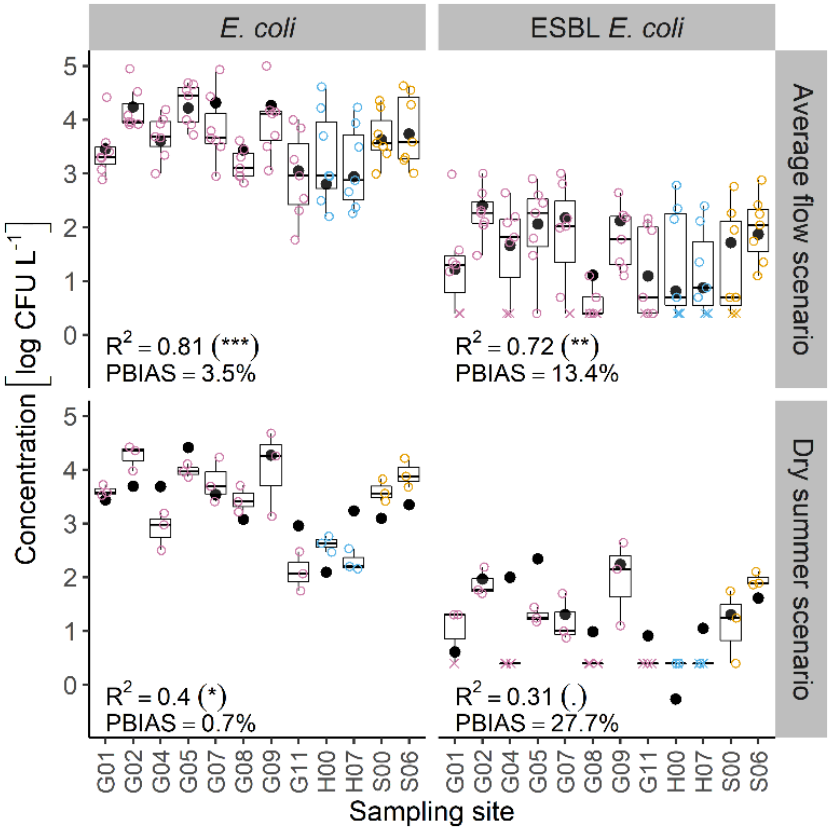

Figure 6.3: Measured and predicted concentrations of *E. coli* and ESBL *E. coli* bacteria in surface water at general catchment sites and longitudinal profiles (upstream and most distant point of longitudinal profile). Measured concentrations are displayed as box-whisker plots and predicted concentrations at steady state as solid black dots. Crosses indicate outliers. Symbols in brackets indicate significance levels of R^2: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, . = $p \geq 0.05$.

6.3.5. Human exposure assessment in the Vecht catchment

Human exposure assessment in the Vecht catchment is conducted only for potential swimming sites (average depth $\geq$ 0.5 m). This includes 44% of cumulated flow length in the average flow scenario. In the dry summer scenario, river depth is generally lower and only waterbodies downstream of WWTPs are deep enough for bathing, making up 26% of cumulated flow length. Result maps for both scenarios are presented in Figures C.4–C.6. At the potential swimming sites predicted *E. coli* concentrations range from less than 1 up to 113 000 CFU L^{-1} for both scenarios. Swimming in fecally contaminated water poses a risk of becoming infected by ingesting fecal pathogens such as the human norovirus (Boehm et al., 2018). High *E. coli* concentrations indicate an increased risk of such an infection. The threshold value for a good bathing water quality (10 000 CFU L^{-1}) laid down in the EU Bathing Water Directive is exceeded by predicted concentrations in only 6% and 7% of potential swimming waters for the average flow scenario and the dry summer sce-

nario, respectively. Exceedance occurs mainly in rivers, where microbial loads emitted by WWTPs are not sufficiently diluted by the receiving waters. The overall contribution of *E. coli* emissions from WWTP effluents to total *E. coli* emissions in the Vecht catchment was 76% and 71% in the average flow scenario and the dry summer scenario, respectively. Due to the emission of *E. coli* by consecutive WWTPs within the river network predicted concentrations can exceed the threshold over up to 20 km flow length. This underlines the importance of WWTPs as source of fecal contamination not only locally but also on the catchment scale. However, diffuse sources can be locally important as the impact of WWTP emissions decreases with increasing distance to the discharge point (Appendix C, Text 5).

Predicted ESBL-EC concentrations in the Vecht catchment were approximately two to three orders of magnitude lower than *E. coli* concentrations in both scenarios, which is a direct consequence of assuming constant ESBL-EC/*E. coli* ratios for diffuse as well as for WWTP emissions (Appendix C, Texts 3 and 4). WWTP effluent contribution to ESBL-EC emissions in the Vecht catchment was 96% in both scenarios. From the highest predicted ESBL-EC concentrations at potential swimming sites (1 071 CFU L^{-1}) in the average flow scenario, a theoretical uptake of 29 and 19 CFU per swimming event was derived for men and women, respectively. In the dry summer scenario, predicted concentrations reach up to 2 272 CFU L^{-1}, which translates into a higher potential uptake of 61 CFU and 41 CFU per swimming event, respectively.

CP-EC are assumed to be present exclusively downstream of WWTPs. This covers 37% of cumulated flow length in the Vecht catchment in the average flow scenario including the Vecht River and its main tributaries Steinfurter Aa, Dinkel and Regge. In summer, water is pumped into smaller tributaries to avoid them falling dry (Section 6.2.4). As a result, a larger fraction of cumulated flow length (53%) is affected by WWTP emissions in the dry summer scenario. CP-EC concentrations are difficult to quantify in surface waters due to their low concentrations. Modeling enables to estimate human exposure towards CP-EC during recreational activities such as swimming: Exposure to the highest predicted CP-EC concentration of 1.2 CFU L^{-1} (calculated in the dry summer scenario) would amount to an uptake of less than one CFU of CP-EC for both, men and women.

The theoretical human ingestion values however be translated to a public health risk as dose-response relationships of ESBL-EC and CP-EC are lacking (Schijven et al., 2015a).

6.3.6. Model limitations and recommendations for future investigations

Monitoring showed that diffuse input of *E. coli* and probably also of ESBL *E. coli* contribute to the overall contamination. Due to insufficient knowledge on the relative contributions of runoff, remobilization from the sediments and groundwater exchange to bacterial in-stream concentrations, these inputs were modelled with a simplified approach. Targeted investigations of *E. coli* and AR *E. coli* concentrations especially in soil, sediment and the different flow components are required to get further insight for model refinement. Monitoring also revealed that WWTP per capita emission rates of (AR) *E. coli* vary by up to one order of magnitude between different WWTPs, which has also been found in other studies (Ouattara et al., 2011; Servais et al., 2007). The average WWTP emission rates applied in the model simulation proved to deliver a realistic overall picture, but lead to over- or underestimation of local inputs. Investigation of the effect of different treatment steps and technologies on *E. coli* removal could help refining the model.

Sedimentation has a strong impact on predicted bacterial concentrations (Appendix C, Text 5). The model assumes a constant fraction of *E. coli* attached to suspended matter. However, a range of 20%–53.6% has been reported (Table C.10). Partitioning of *E. coli* depends on the particle size (Wu et al., 2019) and the clay content (Pachepsky and Shelton, 2011). The role of the suspended solid concentration has been used in many models to estimate the fraction of attached bacteria (Bai and Lung, 2005; Jiang et al., 2015; Kim et al., 2010; Park et al., 2017). However, these require detailed data about composition of suspended solids in the Vecht catchment throughout the year. The sedimentation process itself depends on the settling velocity of the particles, where values between 5×10^{-2} and 1.05 m h^{-1} have been reported (Pachepsky and Shelton, 2011).

The GREAT-ER model provides a realistic picture of the spatial concentration distribution across the catchment for standard flow scenarios, but does not capture concentration variability visible from the monitoring data, since it assumes temporal steady state. Thus, it does also not represent i) the short-term effect of event-driven inputs such as surface runoff or CSO events and ii) natural variability of model parameters. E.g. *E. coli* inactivation rates derived from 95% of in-stream temperatures in the Vecht catchment (2.8–21.9 °C) lead to inactivation rates between 1.4×10^{-2} and 4.7×10^{-2} h^{-1}. However, the model allows for identification of locations of higher-than-average risk. Natural variability and uncertainty of input parameters can be considered in GREAT-ER by applying the already implemented stochastic Monte Carlo simulation routine. An appropriate representation for Monte Carlo simulations is currently parameterized in a German sub-catchment of the Vecht River. This allows the prediction of expected ranges

of microbial concentrations over the course of a year and to systematically investigate the sensitivity of parameters on predicted concentrations.

6.4. Acknowledgements

We are indebted to the Dutch water boards ‚Waterschap Drents Overijsselse Delta', especially Melanie Kuiper, and ‚Waterschap Vechtstromen', especially Anke Durand-Huiting, as well as the German authorities the ‚Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency'(NLWKN) and the ‚State Agency for Nature, Environment and Consumer Protection'(LANUV) for supporting the current study and providing necessary data, and to all WWTP personnel responsible for the actual sampling of wastewater. This work was supported by the European Regional Development Fund (ERDF) of the European Union under the project MEDUWA Vecht(e) (project number 142118). We thank Alfons Uijtewaal for project management of the overall MEDUWA project, and Bianca de Vries for excellent technical assistance.

Original publication: Science of The Total Environment **868**: 161627 (2023)
available at http://dx.doi.org/10.1016/j.scitotenv.2023.161627

Geo-referenced simulations of *E. coli* in a sub-catchment of the Vecht River using a probabilistic approach

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Abstract

The proportion of wild swimmers at non-official bathing sites has increased during the Covid-19 pandemic. Bathing water quality at designated sites is monitored through analysis of the concentration of fecal indicator bacteria such as *E. coli*. However, non-official sites are generally not monitored. In a previous work, steady-state modeling of *E. coli* was achieved at catchment scale, enabling a comparison of expected concentrations along an entire catchment for longtime average. However, *E. coli* concentrations can vary over several orders of magnitude at the same monitoring site throughout the year. To capture the temporal variability of *E. coli* concentrations on the catchment scale, we extended the existing deterministic *E. coli* sub-module of the GREAT-ER (geography-referenced exposure assessment tool for European rivers) model for probabilistic Monte Carlo simulations. Here, selected model parameters are represented by probability distributions instead of fixed values. Wastewater treatment plant (WWTP) emissions and diffuse emissions were parameterized using selected data from a previous monitoring campaign (calibration data set) and in-stream processes were modeled using literature data. Comparison of simulation results with monitoring data (evaluation data set) indicates that predicted *E. coli* concentrations well-represent median measured concentrations although the range of predicted concentrations is lightly larger than the observed concentration variability. The parameters with the largest influence on the range of predicted concentrations are flow rate and *E. coli* removal efficiency in WWTPs. A comparison of predicted 90^{th} percentiles with the threshold for sufficient bathing water quality (according to the EU Bathing Water Directive) indicates that year-round swimming at sites influenced by WWTP effluents is advisable almost nowhere in the study area. A refinement of the model can be achieved if quantitative relationships between the WWTP removal efficiency and both, the treatment technologies as well as the operating parameters are further established.

7.1. Introduction

The Covid-19 pandemic spreading out across the world in 2020 was amongst other measures fought by closing of recreational and sports facilities to minimize contact and reduce the infection risk. As a result, this led to an increase of outdoor activities (Schweizer et al., 2021). In the United Kingdom, for example, the interest in swimming in unsupervised

natural waters (wild swimming at sites not designated as bathing sites) rose considerably (Outdoor Swimmer, 2021). In the European Union (EU), quality parameters for officially designated bathing areas are laid down in the EU Bathing Water Directive (BWD, European Union, 2006). These sites are continuously monitored for concentrations of fecal indicator bacteria Escherichia coli (*E. coli*) and intestinal enterococci (European Environment Agency, 2021). Non-designated swimming sites on the other hand, are not mandatorily surveilled. However, it is known that such bathing sites on rivers and canals are frequently used for recreational activities in Germany and the Netherlands (e.g. Falgenhauer et al., 2021; Wuijts et al., 2020).

Fecal contamination of surface waters is caused by discharge from wastewater treatment plants (WWTPs), combined sewage overflows, runoff from manure-fertilized areas, or direct drop-off of feces by livestock or wildlife (van Heijnsbergen et al., 2022). Such contaminated waters can contain pathogenic viruses, bacteria and protozoa (Boehm and Soller, 2012). Numerous outbreaks of gastrointestinal illnesses (such as diarrhea) associated with exposure to fecally contaminated waters during swimming have been reported (Hall et al., 2017; Parkkali et al., 2017; Wade et al., 2006).

Often, across whole watersheds, information on sites with particular high risks of fecal contamination is lacking. This in turn is needed to evaluate risks at specific non-designated bathing sites. This problem can be overcome by the use of geo-referenced simulation models. Such spatially explicit models can help to identify potential hotspots, to assess local infection risks and to evaluate management options (e.g. O'Flaherty et al., 2019; Schijven et al., 2015b).

In a recent study, the GREAT-ER (geography-referenced exposure assessment tool for European rivers) model, which was originally developed to predict and assess in-stream concentrations of down-the-drain chemicals on the catchment scale (Feijtel et al., 1998; Lämmchen et al., 2021c), had been successfully adapted to simulate the fate of (antibiotic-resistant) *E. coli* (van Heijnsbergen et al., 2022). The model was applied in a case study in the Dutch-German cross-border catchment of the Vecht River in two distinct deterministic scenarios representing average flow situations and typical dry summer situations, respectively (van Heijnsbergen et al., 2022). Such deterministic simulations, however, are not capable of capturing the observed variability of concentrations resulting from variations in flow rate, emissions and fate processes. For example, *E. coli* concentrations in rivers have been reported to range over several orders of magnitude at one single sampling site (Blaak et al., 2014; Ouattara et al., 2011; van Heijnsbergen et al., 2022). Thus, model simulations should not only depict spatial differences, but also capture temporal variability. The probabilistic simulation routine of the GREAT-ER model offers the possibility to assess the range of expected concentrations at single sites in good time. By means of

the Monte Carlo method, parameter variability and uncertainty can be considered in the simulations.

Therefore, in this study the GREAT-ER model is applied for achieving the following objectives: First, the *E. coli* sub-module is parameterized to predict the range of spatially explicit *E. coli* concentrations in the selected sub-catchment of the Vecht River by stochastic modeling. Secondly, we evaluate the impact of variable and uncertain model parameters on the range of predicted concentrations and compare the outcome to the measured variation. Finally, we conduct a first tier hazard assessment for swimming at non-designated bathing sites by evaluating simulated *E. coli* concentrations against threshold values defined in the EU Bathing Water Directive.

7.2. Materials and methods

7.2.1. Study area and hydrological representation

The study area is a sub-catchment of the Dutch-German Vecht River. Large parts of the Dutch sub-catchment include a complex system of canals, which are controlled by pumps in order to keep water levels constant and prevent tributaries from falling dry. These hydrological conditions cannot be represented by the usually applied probability distributions for the Monte Carlo simulations (Lämmchen et al., 2021b). However, in the upstream areas of the catchment - especially in the German tributaries - the river network is in a more natural state, which enables probabilistic simulations in this part. This sub-catchment covers 22% (1 300 km^2) of the whole Vecht catchment and comprises of the first 74 km flow length of the Vecht River, including its main tributaries Dinkel and Steinfurter Aa (Figure 7.1). The 22 WWTPs (4 Dutch, 18 German) in the sub-catchment area treat the wastewater of roughly 300 000 inhabitants. All treatment plants are equipped with conventional activated sludge treatment with the exception of the Ootmarsum WWTP, which uses a membrane bioreactor (MBR) hybrid system - i.e. up to 50% of wastewater is treated by an MBR. None of the WWTPs in the area is equipped with additional disinfection systems for wastewater treatment. The catchment area is characterized by a high proportion of agricultural use (75%) consisting of arable land and pasture.

The river network has previously been set up for simulations in GREAT-ER and has been successfully evaluated for the deterministic simulation of pharmaceuticals (Duarte et al., 2021; Lämmchen et al., 2021b) and (antibiotic-resistant) *E. coli* in the whole Vecht catchment (van Heijnsbergen et al., 2022). To account for the natural variability of river flow, flow rates in GREAT-ER are assumed to be lognormal distributed (Boeije

et al., 1997) with mean annual flow rates taken from Lämmchen et al. (2021b) Standard deviations of flow rates are estimated assuming that the mean annual 10-day minimum flow (MAM10) - derived in the GREAT-ER pre-processing routine - corresponds to the 15th percentile of the long-term probability distribution. This value is similar to MAM10-percentiles reported by (Wissing, 2010) for three German catchments (Ruhr, Saale, Sieg). For each simulation run, a single flow percentile is generated from a uniform distribution between 0 and 1, which is applied to all river segments (Boeije et al., 1997).

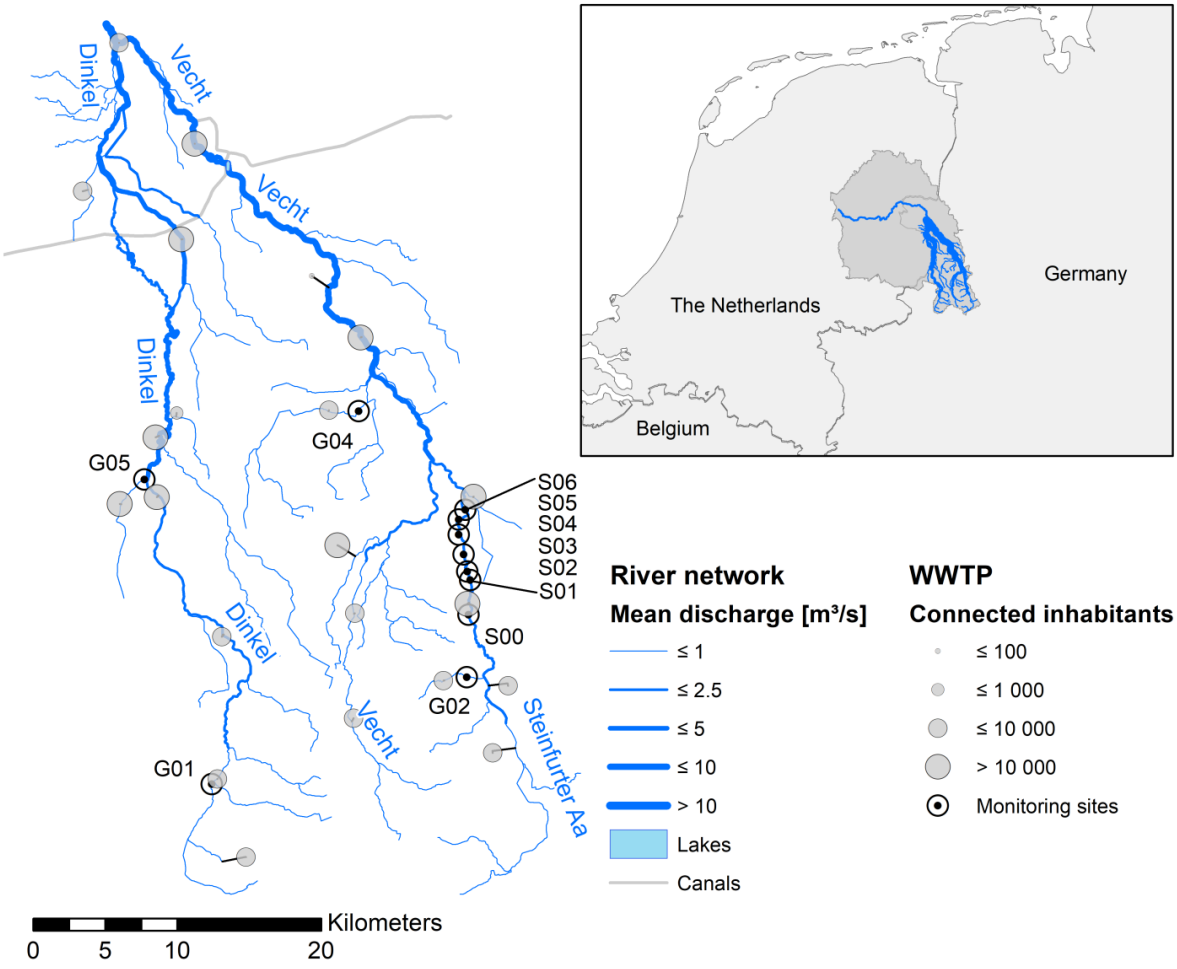


Figure 7.1: Overview of the study area. Monitoring sites are subdivided into "general catchment" sites (G01–G05) and "longitudinal profile" sites (S00–S06). The monitoring campaign was performed by (van Heijnsbergen et al., 2022)

7.2.2. *E. coli* model

The *E. coli* model is based on the deterministic approach presented in van Heijnsbergen et al. (2022) and is adopted to additionally reflect variability in hydrological parameters,

emission and fate throughout the year by means of Monte Carlo simulations. While model equations are the same as in the original model (Table 7.1), selected parameters are described by probability distribution functions instead (Table 7.2).

The *E. coli* model consists of two emission modules, i.e. WWTP emissions and diffuse emissions. The latter encompasses diffuse emissions by runoff and soil leaching, input by wildlife and resuspension and remobilization of *E. coli* from bottom sediments. Local diffuse emissions are calculated by multiplying the flow rate increment of adjacent river segments, i.e. water entering the river segment from local sources, with an aggregated *E. coli* concentration summarizing these diffuse emissions. In-stream processes (inactivation and sedimentation) are modeled as first order loss processes. Sedimentation occurs exclusively for bacteria associated to suspended particles. The share of *E. coli* associated to suspended particles (f_s) is kept constant within a simulation run. Inactivation is simulated as temperature dependent for both, free floating and particle associated *E. coli*. For the latter, the inactivation rate was reduced to 50% of the rate applied to free floating bacteria.

Table 7.1: Model equations of the *E. coli* GREAT-ER model (van Heijnsbergen et al., 2022).

Process	Equation	Parameters
WWTP emission	$L_{eff,i} = pcL \times Inh_i \times 10^{-logRed}$	L_{eff} [CFU yr^{-1}]: *E. coli* effluent load of WWTP i pcL [CFU cap^{-1} yr^{-1}]: *E. coli* per capita emission rate in the influent of WWTP i Inh [cap]: Number of inhabitants connected to WWTP i $logRed$ [-]: Reduction parameter of *E. coli* during the wastewater treatment process
Diffuse emission	$I_x = \Delta Q_x \times C_{diff} \times 1\ 000 \times 31\ 536\ 000$	I_x [CFU yr^{-1}]: Diffuse emission load of river segment x ΔQ_x [m^3 s^{-1}]: Flow rate increment between river segments x and $x-1$ C_{diff} [CFU L^{-1}]: Aggregated *E. coli* concentration in flow rate increments 1 000: Conversion from m^3 to L 31 536 000: Conversion from yr to s
Sedimentation	$k_{set} = \frac{v_{set}}{d_x}$	k_{set} [h^{-1}]: First order settling rate v_{set} [m h^{-1}]: Settling velocity of suspended particles d_x [m]: Depth of river segment x
Inactivation	$k(\vartheta) = k_{20} \times \frac{\exp\left(\frac{-(\vartheta-25)^2}{400}\right)}{\frac{-25}{400}}$	$k(\vartheta)$: Temperature dependent first order in-stream inactivation rate ϑ [°C]: Temperature k_{20} [h^{-1}]: Inactivation rate at 20 °C

7.2.3. Stochastic parameterization

In probabilistic simulations, input parameters are represented by probability distributions (Table 7.2). Parameterization was based on literature values (adsorbed fraction, settling velocities), model calculations (flow rate, flow rate increment) and a one year monitoring campaign in the Vecht catchment (per capita influent load, log reduction in WWTPs, flow rate increment concentration) performed by van Heijnsbergen et al. (2022). Some model parameters were treated as being correlated with each other. Technically, we correlated the percentiles of the respective parameter distributions to generate the dependent values.

Table 7.2: Simulation parameters for probabilistic *E. coli* simulations.

Parameter	Parameter name	Distribution	Mean	Standard deviation	Median	Unit
Per capita influent load	pcL	lognormal	9.14×10^{12}	6.75×10^{12}	7.35×10^{12}	CFU cap^{-1} yr^{-1}
Log reduction in WWTP	$logRed$	normal [a]	2.72	0.90	2.72	–
Temperature	ϑ	normal [b]	11.9	5.5	11.9	°C
Adsorbed fraction	f_s	beta	35.6	9.0	35.2	%
Settling velocity	v_{set}	lognormal	0.14	0.12	0.1	m h^{-1}
Flow rate	Q	lognormal [c]	Q_{mean}	Q_{sd}	Q50	m^3 s^{-1}
Flow rate increment concentration	C_{diff}	lognormal	5.0×10^3	8.3×10^3	2.6×10^3	CFU L^{-1}

[a] Values smaller than 0 and higher than 5.44 are omitted to ensure positive values and symmetry.

[b] Values smaller than 0 were excluded from simulations.

[c] Flow rate Q is lognormally distributed for each segment in the river network with mean flow rate Q_{mean} and standard deviation Q_{sd}. Q50 is the respective median of calculated flow rates.

Per capita influent loads of *E. coli* were calculated from measurements of influent concentrations in ten WWTPs (monthly samples, nine to ten samples per WWTP) in the Vecht catchment by multiplication with the actual discharge and subsequent normaliza-

tion with the number of inhabitants connected to the WWTP (van Heijnsbergen et al., 2022). Statistical analysis showed that the variability of the per capita loads could be well described by a lognormal distribution (Kolmogorov-Smirnov Test, $p < 0.001$).

Reduction of *E. coli* in WWTPs during the wastewater treatment process is expressed by a log reduction parameter (*logRed* [-], see Table 7.1). This parameter was derived as the log ratio of measured influent (C_{in} [CFU L^{-1}]) and effluent concentrations (C_{eff} [CFU L^{-1}]):

$$logRed = -\log\left(\frac{C_{eff}}{C_{in}}\right) \tag{7.1}$$

The treatment process *logRed* is expressed as normal distributed function (2.72 ± 0.90, Kolmogorov-Smirnov Test, $p < 0.001$) and it reflects the variance between and within WWTPs, as different WWTPs were sampled at different dates. By definition, *logRed* is zero for no reduction so smaller values were excluded by cutting off the distribution at the respective percentile ($P = 1.26 \times 10^{-3}$). For the sake of consistency in terms of symmetry around the mean, the distribution was also cut off at the respective upper end at $logRed = 5.44$. Recent studies have shown that the treatment efficiency for *E. coli* is lower within the same WWTP with increasing discharge due to reduced hydraulic retention times (Pallares-Vega et al., 2021; van Heijnsbergen et al., 2022). In combinated sewer systems, WWTP discharge increases with rainfall (Mines et al., 2007), which also affects river flow (Bormann, 2010; Jiang et al., 2007; Pourfallah Koushali et al., 2021). Therefore, we used flow rates in receiving river segments as proxy for WWTP discharge and determined its correlation with *logRed*. The resulting moderate correlation ($\rho = -0.41$) was implemented into the stochastic model for the WWTPs.

Modeled diffuse emissions to a river segment depend on two parameters, namely the flow rate increment of the river segment (ΔQ_x) and the *E. coli* concentration in this increment (C_{diff}, see Table 7.1). The flow rate increment of a river segment x is defined as the difference of its flow rate Q_x to the flow rate of adjacent upstream river segments. Flow rate increments are parameterized as lognormal distributions excluding unrealistic negative values. For lack of explicit data, we assume that the coefficient of variation of a flow rate increment is the same as for the flow rate of the corresponding river segment. Within a simulation run, the flow rate increment percentile is assumed strongly correlated ($\rho = 0.8$) with the flow rate percentile. The *E. coli* concentration for flow rate increments was derived from measured concentrations of seven sampling sites without any known WWTP influence (10 monthly samples each) in the Vecht catchment (van Heijnsbergen et al., 2022) under the assumption of a lognormal distribution.

Consideration of temperature variability for bacteria inactivation makes use of measured in-stream temperatures at different monitoring sites in the Vecht catchment for the years 2008–2019. Data were best represented by a normal distribution (11.9 ± 5.5 °C, Kolmogorov-Smirnov Test, $p < 0.001$). We excluded values below the freezing point of water (0 °C). The inactivation rate at 20 °C is the same (4.5×10^{-2} h^{-1}) as in van Heijnsbergen et al. (2022), adopted from Ouattara et al. (2013).

The fraction of *E. coli* attached to suspended particles (f_s) is adopted from data of studies in other catchments (mean = 35.6%, sd = 9.0%) (Table D.1) and expressed as beta distribution to ensure that parameter values are restricted to the interval [0, 1].

The settling velocity of suspended particles depends on its size and density (Jamieson et al., 2005a; Wang et al., 2018) as well as on the turbulence of the water (Murray, 1970; Nielsen, 1993). For suspended matter to which *E. coli* adsorb, a wide range of values has been reported in the literature (Table D.2). To map this variability, we express the settling velocity by a lognormal distribution. The median was set equal to the value applied for deterministic simulations of *E. coli*, i.e. 0.1 m h^{-1} (van Heijnsbergen et al., 2022), adopted from Ouattara et al. (2013). A standard deviation was chosen that makes sure the 90% percentile range (P_5–P_{95}) covers existing literature values.

7.2.4. Monte Carlo simulations

Model convergence

Stochastic simulations are performed using the Monte Carlo approach. The simulation parameters are represented by probability distributions. In the Monte Carlo simulation, deterministic runs are repeatedly performed. In each realization, a random value is drawn from the input distributions of each simulation parameter (Boeije et al., 1997). For Monte Carlo simulations of down-the-drain chemicals with GREAT-ER, 1 000–50 000 simulation runs are recommended for achieving stable mean output values (Kehrein et al., 2015; Schowanek and Webb, 2002; Schulze, 2001). We performed 100 000 runs for each scenario which led to deviations of less than 5% of predicted concentrations when a simulation was run twice.

Result parameters

The probabilistic simulation routine of the GREAT-ER model produces spatially resolved concentrations in the form of lognormal distributions. The median of these distributions can be interpreted as follows: If a grab sample is taken at any time and analyzed for

E. coli, the chance is 50% that the predicted median concentration is not exceeded, and the chance is 80% that the value is between the 10^{th} and 90^{th} percentile.

7.2.5. Analysis

Analysis of model performance

Predicted concentration distributions are compared to measured concentrations at monitoring sites across the catchment (Figure 7.1). A detailed description of the one-year sampling campaign (2018/2019) is provided in van Heijnsbergen et al. (2022). These sites do not include the monitoring sites used for the parameterization of the model. Monitoring sites are subdivided into general catchment sites (G01–G05) and longitudinal profile sites (S00–S06). The latter are located in the Steinfurter Aa with five sampling sites downstream and one site upstream of the WWTP S. All sampling sites used for the evaluation are downstream of at least one WWTP discharge point.

For numerical comparison of predicted and measured concentrations, we assume that concentrations in random samples also obey to a lognormal distribution. We compare the median of measured and predicted concentrations as central moments. To compare the variability of lognormal concentration distributions (predicted vs. measured) we make use of the fact that the width of a certain percentile range of a lognormal distribution only depends on the standard deviation (σ) of the underlying normal distribution (see Appendix D, Text 1 and equation D.7). Therefore, we examine the difference in the standard deviations of predicted and measured concentrations ($\Delta\sigma$) at each sampling site:

$$\Delta\sigma = \sigma_{pred} - \sigma_{meas} \tag{7.2}$$

where σ_{pred} and σ_{meas} are the standard deviations of the predictions and measurements, respectively.

If $\Delta\sigma$ is positive, predicted concentrations are more variable then measured ones and vice versa. The difference of a certain percentile range can be read directly from the $\Delta\sigma$ value (see Appendix D, Text 1 and equation D.8), e.g. the log change of the 80%-percentile range (P_{10}–P_{90}) is $1.11 \times \Delta\sigma$. Thus, if $\Delta\sigma$ is 0.9, the 80%-percentile range of predicted concentrations is an order of magnitude (factor 10) larger compared to the 80%-percentile range of measured concentrations.

Impact of parameter distributions on predicted concentration ranges

To evaluate the influence of parameter uncertainty on the range of predicted in-stream concentrations, scenarios were created in which one parameter was kept constant at the median value, while all others were parameterized as probability distributions. Investigated parameters were the per capita emission rate, the logarithmic reduction during wastewater treatment, the in-stream temperature, the adsorbed fraction, the settling velocity, the flow rate, and the *E. coli* concentration in flow rate increments as listed in (Table 7.2).

Analogous to the comparison of concentration ranges during model evaluation, we define the change in the standard deviations of predicted concentrations ($\Delta\sigma$) over all river segments between reference and test scenario as measure for the impact of the test parameter's variability on total variance.

$$\Delta\sigma = \sigma_{ref} - \sigma_{test} \tag{7.3}$$

where σ_{ref} and σ_{test} are the standard deviations for the reference scenario and the tested scenario, respectively. Here, a positive value indicates a decrease in variability and a negative value an increase.

7.2.6. Preliminary exposure assessment

The EU Bathing Water Directive defines concentrations for "excellent", "good" and "sufficient" water quality based upon a 90^{th} or 95^{th} percentile evaluation (Table 7.3). If the target for sufficient bathing water quality is not reached, the bathing water quality is called "poor". The defined values apply to official EU bathing waters - mostly lakes and coastal sites which are continuously monitored (European Environment Agency, 2021). In this study, we focus on *E. coli* as indicator bacteria. In the study area, there is no official bathing site, but a number of non-official bathing sites in the Vecht catchment are used for "wild swimming" (Uijtewaal and Amador, 2021). The evaluation of the bathing water quality is based on samples taken shortly before and during the bathing season. We assume that wild swimming takes place over the entire year.

Table 7.3: Threshold values of the EU Bathing Water Directive for inland waters (Directive 2006/7/EC, European Union, 2006).

Parameter	Excellent quality	Good quality	Sufficient
Escherichia coli [CFU L^{-1}]	5 000 [a]	10 000 [a]	9 000 [b]
Intestinal enterococci [CFU L^{-1}]	2 000 [a]	4 000 [a]	330 [b]

[a] based upon a 95th percentile evaluation

[b] based upon a 90th percentile evaluation

7.3. Results and Discussion

7.3.1. Evaluation of stochastic model

Figure 7.2 presents a graphical comparison between measured and predicted concentrations at six sampling sites across the sub-catchment. Predicted median concentrations slightly overestimate the median of measured concentrations (factor 1.1–2.0) for all sampling sites except for G01 (factor 0.7). Although the monitoring campaign provides a comprehensive picture of *E. coli* concentrations in the sub-catchment across a year, it cannot be excluded that data are biased towards lower concentrations, i.e. by flow conditions or other environmental conditions. All $\Delta\sigma$ values are positive (0.13–1.41) indicating that predicted concentrations are larger than measured ones. This is corroborated by the fact that 97% of measured concentrations are within the 80% percentile range of the predicted local concentration distributions. The effect of variability in model parameters on the predicted concentration variance will be discussed further in section 7.3.2.

The 80th percentile of predicted concentrations covers all measured concentrations along the Steinfurter Aa (Figure 7.3). The graph illustrates the longitudinal concentration profile of the Steinfurter Aa including 46 flow kilometers of the Vecht River after the confluence. A local increase in predicted concentrations is caused by an emission source or the confluence with a higher contaminated stream. Figure 7.3 shows that the influence of WWTP S on measured concentrations is captured well by the stochastic model. WWTP emissions lead to an increase of predicted median concentrations by up to a factor of 6. Despite diffuse emissions along the river course, concentrations decrease with increasing distance to the last WWTP emission due to dilution and dissipation processes, i.e. inacti-

vation and sedimentation. A confluence with a less polluted tributary leads to a decrease in concentration.

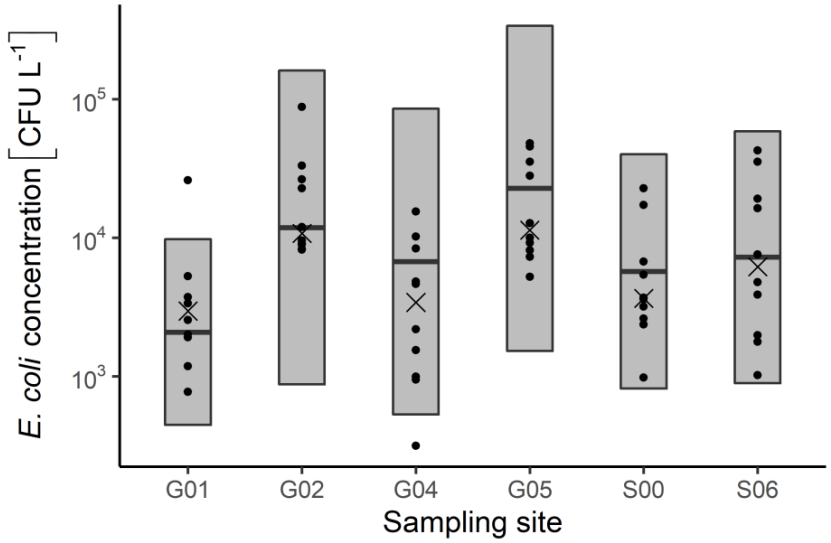


Figure 7.2: Comparison of predicted and measured concentrations in the catchment. Boxes indicate the range between the 10th percentile and the 90th percentile of predicted concentrations. Horizontal lines indicate median predicted concentrations. Dots indicate measured concentrations; crosses represent the local median value. Monitoring site S06 is representative for the six monitoring sites in the downstream profile of WWTP S.

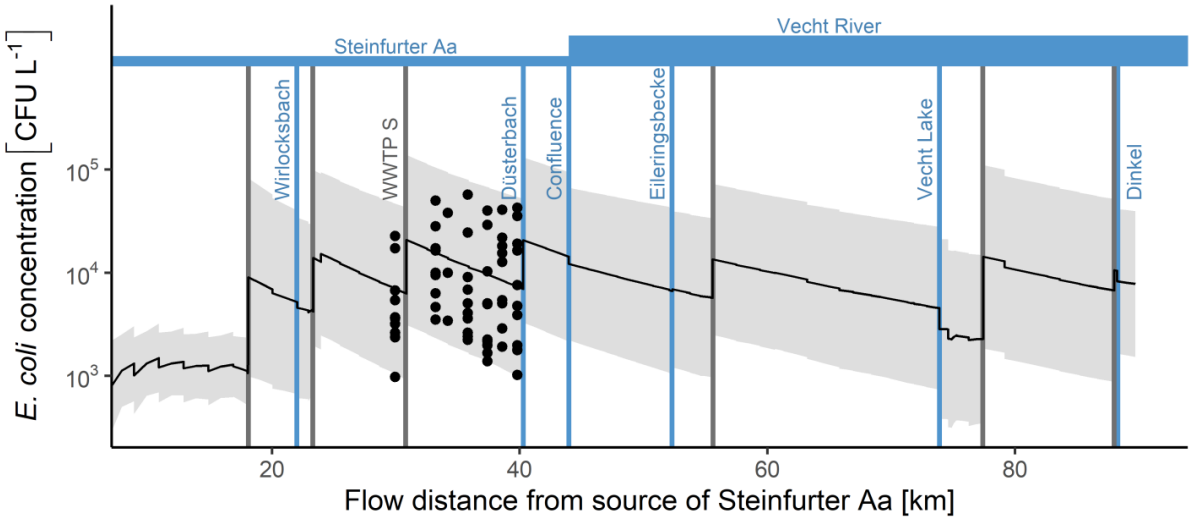


Figure 7.3: Longitudinal concentration profile of the Steinfurter Aa and the Vecht River after confluence. Dots indicate measured concentrations. The solid line indicates the median of predicted concentrations and the shaded area the range between the 10th percentile and the 90th percentile. Important tributaries (and confluences) and wastewater treatment plant (WWTP) emissions are indicated by vertical blue and grey lines, respectively.

7.3.2. Impact of parameter distributions on the predicted concentration range

In the evaluation of the impact of model parameter uncertainty on the variance of predicted *E. coli* concentrations, it was distinguished between river segments upstream and downstream of WWTPs, since the two WWTP related parameters (*logRed*, *pcL*) only affect the latter. For the dissipation processes, variability of temperature and of the adsorbed fraction have limited influence on the predicted variability, i.e. $\Delta\sigma$ values are close to zero (Figure 7.4). The parameterization of the settling velocity also has a minor effect on the variability of predicted concentrations (median of $\Delta\sigma$ values is 0.05).

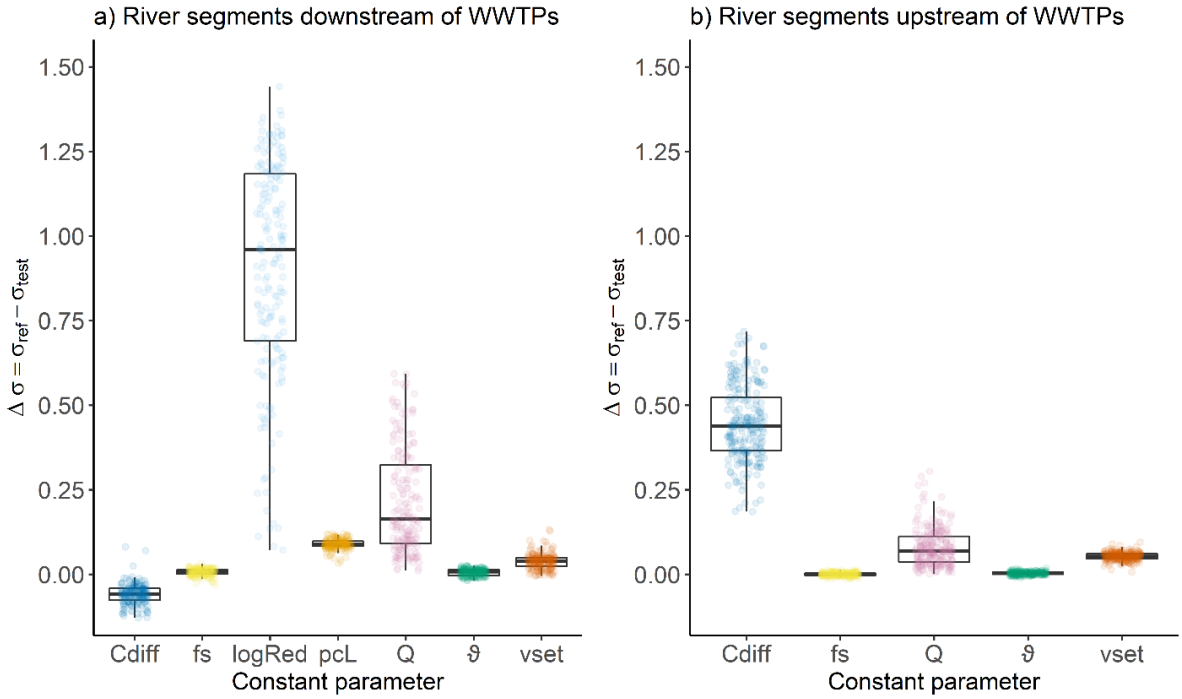


Figure 7.4: Effect of variation in one parameter on variation of overall river concentrations, shown as the difference of standard deviations ($\Delta\sigma$) from the reference scenario (σ_{ref}) and test scenarios (σ_{test}), in which one parameter was kept constant. Test parameters include C_{diff} (flow rate increment concentration), f_s (adsorbed fraction), *logRed* (log WWTP reduction), *pcL* (per capita load in WWTP influent), Q (river flow rate), ϑ (temperature), v_{set} (settling velocity). River segments are distinguished in (a) downstream and (b) upstream of wastewater treatment plants (WWTPs). Each data point represents the $\Delta\sigma$ value for one river segment. Boxes correspond to the 25$^{\text{th}}$ and the 75$^{\text{th}}$ percentiles. Whiskers are limited to a maximum of 1.5 times the interquartile range.

Positive $\Delta\sigma$ values higher than 0 indicate a decrease oflower variance in the test scenario compared to the reference scenario and negative $\Delta\sigma$ values are indicative for larger variance. For river segments downstream of WWTPs the reduction parameter (*logRed*) appears to be the most influential parameter for the overall variance (Figure 7.4a). This

is not surprising, since the 80%-percentile range (P_{10}–P_{90}) of *logRed* spans three orders of magnitude (*logRed* 1.2 to 4.2). The high variability in the modeled treatment efficiency arises from the representation of both the variability within a WWTP and the variability between WWTPs. Temporally variable *E. coli* removal efficiency within the same WWTP has been reported by Barrios-Hernández et al. (2020) who found *logRed* values of 1.12 ± 0.69 and 1.65 ± 0.68 at two WWTPs in winter and spring, respectively. The variability of the reduction between WWTPs is depending on primary treatment, the type of CAS treatment and nutrient removal (Ouattara et al., 2011; Raboni et al., 2016). Blaak et al. (2021) studied 100 different full-scale WWTPs and reported a 90% confidence interval for log reduction of 0.77–5.9, which clearly reveals the large inter-WWTP variability. The contribution of the per capita load parameter turned out to be much smaller compared to the reduction parameter. Not only the median of the $\Delta\sigma$ values (representing the general tendency), but also its variability (representing the difference in local impact) is much smaller for *pcL* than for *logRed* (see Figure 7.4).

The impact of parameter distributions on the variability of predicted concentrations at waterbodies affected by wastewater was second highest for the flow rate (Figure 7.4a). The flow rate has a direct effect on the concentration via dilution but additionally exerts indirect effects, since it determines the flow velocity and the depth of a river segment, all increasing the variability of predicted concentrations. The flow rate has a comparably weaker impact on river segments upstream of WWTPs (Figure 7.4b). This results from the strong correlation between flow rate and discharge increment. The variability of predicted concentrations at these sites rather depends on the theoretical concentration in the flow rate increments. Keeping this parameter constant led to a median decrease of the 80%-percentile range by factor 3. On the contrary, at river sites downstream of WWTPs the constant concentration in the flow rate increments led to positive $\Delta\sigma$ values, indicating that diffuse emissions have a buffering effect on the range of predicted concentrations. However, this effect is comparably small; i.e. the median of the $\Delta\sigma$ values across all segments is -0.06.

7.3.3. Preliminary exposure assessment

Figure 7.5 illustrates the 90^{th} percentile of predicted *E. coli* concentrations. A concentration higher than 9 000 CFU L^{-1} based on a 90^{th} percentile evaluation indicates poor bathing water quality according to the Bathing Water Directive. The 90^{th} percentile of predicted *E. coli* concentrations ranges from 2×10^3 to 1×10^6 CFU L^{-1}. It is assumed that only sites downstream of WWTPs provide a sufficient water depth for year-round swimming (van Heijnsbergen et al., 2022) A concentration of 9 000 CFU L^{-1} is exceeded

at 90% of cumulated flow length of these waterbodies which suggests that these sites are not suitable for year-round swimming. This statement is also qualitatively consistent with the results of the monitoring campaign. The 90th percentile of measured concentrations exceeds a concentration of 9 000 CFU L^{-1} at all monitoring sites downstream of WWTPs in the study area (see Figure 7.5).

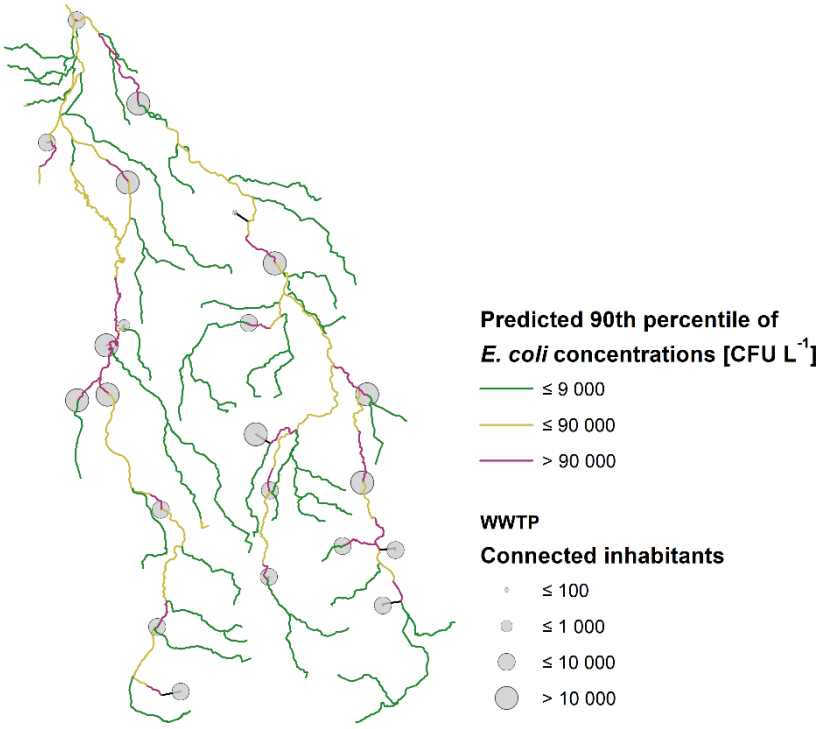


Figure 7.5: 90th percentile of predicted *E. coli* concentrations. A concentration higher than 9 000 CFU L^{-1} based on a 90th percentile evaluation marks a poor water quality (Directive 2006/7/EC).

It is important to keep in mind that the range of predicted *E. coli* concentrations does not consider short-term concentration peaks caused by heavy rain events, because the steady-state model does neither cover sewage overflow (Passerat et al., 2011) nor peak runoff from agricultural areas (Ling et al., 2009) treated with manure (Harmel et al., 2010; Meals and Braun, 2006).

7.4. Conclusions and perspectives

The extension of the existing deterministic approach to simulate *E. coli* in whole catchments renders the determination of the range of expected concentrations possible. Additionally, it allows for categorization of parameters according to their influence on the variability of predicted concentrations. While the deterministic approach only offered

predictions for average flow situations, the extended model now enables a more comprehensive assessment throughout the year. Furthermore, the model helps identifying bathing sites with low infection risk.

While wild-swimming takes places the entire year, activities increase especially in summer. Further model applications therefore could focus on the bathing water season. However, the current implementation of the GREAT-ER model does not allow for a probabilistic parameterization of hydrological conditions in summer due to missing season-specific input data. Current GREAT-ER simulations are based on the steady-state approach and do thus not consider event-driven concentration peaks, which can overlay simulated annual average concentrations.

The most influential model parameters on predicted concentration ranges are the WWTP removal efficiency and the flow situation. The parameterization of the flow rate for the GREAT-ER model has already been proven appropriate to describe the flow rate over the year in studies on micropollutants in various catchments (e.g. Kehrein et al., 2015; Lämmchen et al., 2021c; Schowanek et al., 2001). To enhance the reliability of *E. coli* simulations in catchments without experimental data on WWTP performance, the fate of *E. coli* during wastewater treatment as depending on boundary conditions of the WWTPs (e.g. operating parameters, daily discharge) needs to be investigated in more detail.

In a next step, the model can be applied to antibiotic-resistant bacteria like extended spectrum beta-lactamase (ESBL) producing *E. coli* or carbapenemase-producing *E. coli*. Schijven et al. (2015a) used quantitative microbial risk assessment (QMRA) models to calculate probabilities for the uptake of antibiotic-resistant bacteria during swimming based on local concentrations. By coupling GREAT-ER simulation results with QMRA models, these uptake probabilities could be determined on the scale of an entire catchment.

7.5. Acknowledgements

This work was financially supported by the European Regional Development Fund (ERDF) of the European Union within the INTERREG V-A programme Deutschland-Nederland under the project MEDUWA-Vecht(e) (project number 142118).

8. GREAT-ER software extensions

8.1. GREAT-ER modules

In the latest GREAT-ER version (GREAT-ER 4.0 Kehrein et al., 2015), the user selects a workspace (a database) in the first step. The database stores simulation data, including compound data and scenario definitions (Kehrein et al., 2015). To perform a simulation a scenario is loaded into a session. Within a session the user can edit a scenario via the user interface, e.g. chose submodels or change simulation parameters.

The GREAT-ER software was extended to allow for the simulation of surface water exposure to bacteria with special focus on antibiotic-resistant species. The approach was then exemplary applied to *E. coli* and ESBL *E. coli* in the Vecht catchment (Chapters 6 and 7). The simulation of bacteria required some general adaptations of the simulation tool. The necessity is most evident from the different unit systems used for chemicals (masses, kg) and bacteria (number of individuals or colony forming units, CFU). Similarly, not all sub-models required for the simulation of chemicals are also useful for the simulation of bacteria, and vice versa.

It was decided to introduce a modular structure in GREAT-ER for selecting different compound classes (Figure 8.1). When setting up a simulation, the user selects the module, which activates the respective compound class. Within a certain compound class, further differentiation into compound types is possible. The compound type determines the availability of different emission and fate sub-models. In the compound class "bacteria" only one compound type is defined to date, but this can be extended later for example to distinguish between resistant and non-resistant species, if necessary. The compound class "chemicals" on the other hand contains compound types like pharmaceuticals, contrast agents, or dissolved metals. Depending on the selection of the compound class (and the compound type), the user interface presents corresponding data and the user is prompted for input of required parameters of activated sub-models.

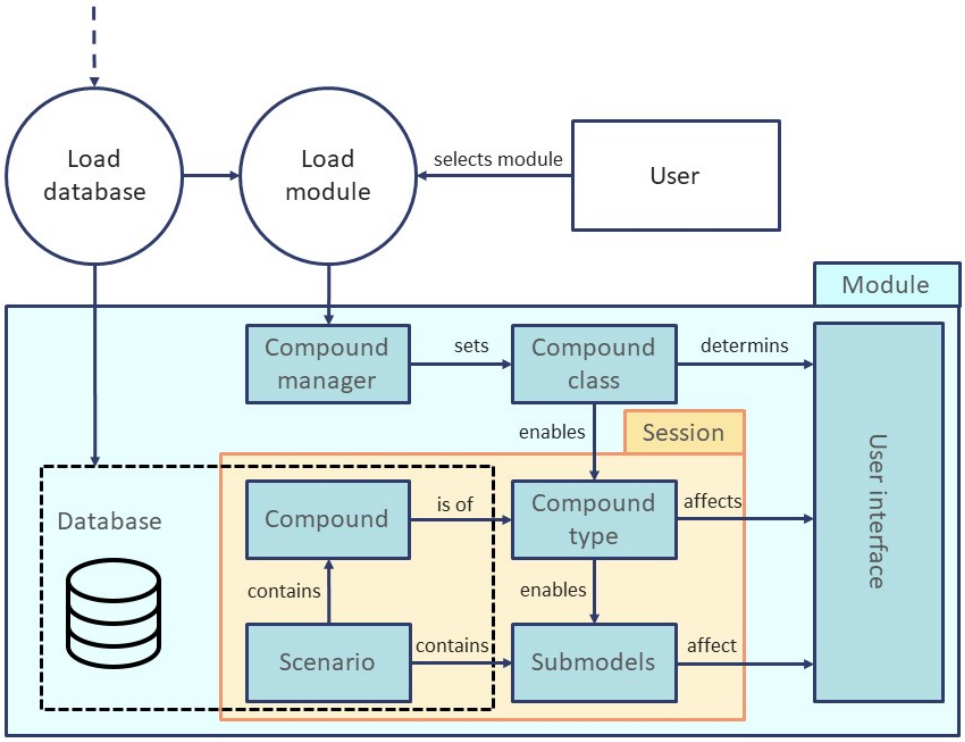


Figure 8.1: Conceptual design of GREAT-ER modules.

8.2. Update of GREAT-ER emission sub-models

8.2.1. Hospital input model

Pharmaceuticals represent a compound type that is predestined to be simulated with GREAT-ER. Since some of these substances are largely administered in hospitals (see Chapters 4 and 5), the general per capita assumption does not fully capture the spatial distribution of their emissions. In GREAT-ER, the sub-model "hospital input" can be selected for pharmaceuticals, which enables the model to treat the emission with hospital wastewater separately. Therefore, the user has to define the total amount (or fraction of total use volume) of the substance that is administered in hospitals or specialized private practices. For example, for X-ray contrast agents this fraction is close to 100%.

This sub-model has been adapted during this thesis to allow for more transparent and more detailed input figures. It still requires a proxy for the number of treatments per location, which can be the number of hospital beds or the number of treated inpatients. Emissions are then defined as administered amount per beds or inpatients. This number can be specified individually for each hospital or as average value to be applied to all (other)

hospitals in the area. Thus, depending on the spatial resolution of available consumption or sales figures (e.g. aggregated for countries, federal states, municipalities or individual hospitals), the model can now be parameterized to represent the respective level of detail. This is especially useful for pharmaceuticals, which are preferably dispensed in specialized clinics or departments. An exemplary application of this feature is included in Chapter 4. It has to be notified that the user is responsible for a plausibility check: The total amount of the pharmaceutical administered in the area must always equal the sum of all individual applications.

8.2.2. Country-specific emission assumptions

This sub-model allows for country-specific parameterization of domestic and/or hospital emission. If selected, the respective emission parameters (per capita use and hospital consumption / per patient application) can be differentiated by country. In the database, countries are specified by name and a unique ID, which is then assigned to all WWTPs and hospitals in the area. This allows for regionally different emission parameterization in GREAT-ER. As an example, the second research article describes the application of this feature for selected pharmaceuticals in the cross-border catchment of the Vecht River (located in the Netherlands and Germany), whose consumption is different in the two countries (Chapter 5).

8.2.3. Diffuse emissions

The simulation of *E. coli* made it necessary to account for diffuse emission sources (Chapters 6 and 7). The existing approach implemented for the diffuse emissions of copper and zinc (Hüffmeyer et al., 2009) defines diffuse input loads as dependent from the area of the sub-catchment which is assigned to a river segment. For *E. coli*, however, diffuse emissions (consisting of remobilization from sediment, groundwater input, input via interflow, and surface runoff, see Chapter 6) depend rather on river flow rates and contributing flow components; i.e. groundwater, interflow and runoff. The GREAT-ER model does currently not distinguish between these three flow components. They are aggregated in a single discharge increment between two river segments. To account for the diffuse input of *E. coli*, a simulation routine was implemented where a theoretical bacterial concentration can be assigned to discharge increments. For different flow situations different concentrations can be defined, e.g. average flow and low flow in (Chapter 6).

8.3. Model evaluation tools

A model evaluation tool was developed to facilitate visualization of the results with focus on the comparison of predicted concentrations against measured concentrations. For this purpose, calculation of different model evaluation metrics and a number of meaningful graphical outputs were implemented.

8.3.1. Model evaluation metrics

Evaluation metrics are used to compare model outputs (e.g. mean or median of predicted concentrations) with respective concentration measurements at the same site. In deterministic simulations, a single concentration is predicted for a river segment representing an average situation, while there might be more than one measured value for the respective sampling site. Therefore, for some metrics, measured values are aggregated per monitoring site and the median is used. Table 8.1 provides an overview of the implemented metrics and their specific meaning.

The simplest and easy-to-understand evaluation metric is the percentage of deviations by more than a factor 3 or factor 10 of all pairs of values (PEC, MEC), which are common measures of model quality. The fraction of predictions deviating less than a specified factor from observed data is a readily accessible but not very expressive for centrality. Factor 10 defines whether the model output is of the same order of magnitude as the measurement, which is often considered as sufficient agreement for data varying over several orders of magnitude such as bacteria counts. Sometimes this magnitude factor is defined as square root of ten, which is approximately three.

Other widely established metrics in model evaluation are the root mean square error (RMSE) and the coefficient of determination (R^2). R^2 has been widely used to evaluate the performance of water quality models. It describes the share of the variance in the observed data, which can be explained by the model. R^2 values range from 0 to 1 with high values indicating less error variance. However, this metric is sensitive towards outliers (Moriasi et al., 2007; Singh et al., 2004). RMSE is another commonly used error index (Moriasi et al., 2015). This statistic penalizes errors quadratically. Values are always greater or equal to 0, with 0 indicating no error (Singh et al., 2004). R^2 and RMSE were also implemented for log-transformed data to address the sensitivity towards outliers and evaluate model performance on the log scale.

Table 8.1: Model evaluation metrics implemented in the GREAT-ER software. x: predicted environmental concentration, y: measured environmental concentration, r_i: y_i/x_i, i: Index of predicted-measured pairs M: Median, n: number of predicted-measured-pairs, sgn: signum function, m: number of sampling sites, j index of sampling sites, $\tilde{y_j}$ median measured concentration at sampling site j, $\bar{x}$ mean predicted concentration at all sampling sites, $\bar{y}$ mean of median measured concentration at all sampling sites.

Metric	Meaning	Definition	Comment	Application
ξ	Median symmetric accuracy	$100 \times (\exp(\mathrm{M}(\lvert \ln r_i \rvert)) - 1)$	Percentage, robust metric for centrality	Chapter 5
SSPB	Symmetric signed percentage bias	$100 \times (\exp(\lvert \mathrm{M}(\ln r_i) \rvert) - 1) \times \mathrm{sgn}(\mathrm{M}(\ln r_i))$	Percentage, robust metric for over- and underestimation	Chapter 5
Factor 3	Deviations of less than factor 3	$100 \times \frac{1}{n} \left\lvert \left\{ r_i \middle\vert \frac{1}{3} < r_i < 3 \right\} \right\rvert$	Percentage of predicted concentrations differing less than factor 3 from measured concentrations	Chapter 5
Factor 10 (magnitude)	Deviations of less than factor 10	$100 \times \frac{1}{n} \left\lvert \left\{ r_i \middle\vert \frac{1}{10} < r_i < 10 \right\} \right\rvert$	Percentage of predicted concentrations differing less than factor 10 from measured concentrations	Chapter 5
RMSE	Root mean square error	$\sqrt{\frac{1}{m} \sum_{j=1}^{m} (\tilde{y_j} - x_j)^2}$	Quadratic penalty	
R^2	Coefficient of determination	$\left(\frac{\sum_{j=1}^{m} (x_j - \bar{x})(\tilde{y_j} - \bar{y})}{\sqrt{\sum_{j=1}^{m} (x_j - \bar{x})^2} \times \sqrt{\sum_{j=1}^{m} (\tilde{y_j} - \bar{y})^2}} \right)^2$	Proportion of variance in measured data explained by the model, sensitive to outliers	
$\mathrm{RMSE}_{\log}$	Root mean square error of log-transformed data	$\sqrt{\frac{1}{m} \sum_{j=1}^{m} (\ln \tilde{y_j} - \ln x_j)^2}$	Quadratic penalty, more robust than RMSE	Chapter 6
${R^2}_{\log}$	Coefficient of determination of log-transformed data	$\left(\frac{\sum_{j=1}^{m} \left(\ln x_j - \overline{\ln x}\right)\left(\ln \tilde{y_j} - \overline{\ln y}\right)}{\sqrt{\sum_{j=1}^{m} \left(\ln x_j - \overline{\ln x}\right)^2} \times \sqrt{\sum_{j=1}^{m} \left(\ln \tilde{y_j} - \overline{\ln y}\right)^2}} \right)^2$	Proportion of variance in measured data explained by the model, more robust than R^2	Chapter 6

Less known metrics in this context are the median symmetric accuracy (ξ) and the symmetric signed percentage bias (SSPB). Since both are median-based metrics, they are robust towards outliers (Morley et al., 2018). They had been developed to evaluate the performance of models on the log scale. Both metrics build on the relative error (r) between predicted (x) and measured concentrations (y):

$$r = \frac{x}{y} \tag{8.1}$$

ξ is a metric of centrality with values greater or equal to 0. A value of 0% indicates highest centrality of relative errors, i.e. the median relative error is 0. On the other hand, a value of 100% means that half of the data points have relative errors greater than 100% (factor 2). SSPB is a useful metric to quantify overestimation or underestimation of data by the model. A negative value indicates a tendency towards underestimation. A value of -100% means that half of the measured values are underestimated by more than factor 2. Both metrics have been applied to evaluate the ePIE (exposure to Pharmaceuticals in the Environment) model (Oldenkamp et al., 2018) and the predictions of pharmaceutical emissions from hospitals (Zillien et al., 2019). Further examples of ξ and SSPB can be found in Chapter 5.

Figure 8.2 shows the output table of the model evaluation parameters in the GREAT-ER software. In addition to the calculation of metrics for measured and predicted concentrations, a calculation for loads is also possible. This requires information about the river flow at the sampling site on the date of sampling.

Parameter zur Modellevaluierung

ξ	SSPB	Faktor 3	Faktor 10	R^2	RMSE	R^2 log	RMSE log
183%	41%	52%	92%	0,40	6532,6	0,80	0,27

Figure 8.2: Model evaluation parameters in the GREAT-ER software.

8.3.2. Graphical outputs

A first evaluation of simulation results is supported by graphical representation of the data. In this thesis, the software was extended by a number of standard formatted outputs. The user can opt for direct comparison of measured and predicted data at the different sites (Figure 8.3a). A solid line represents perfect agreement (1:1 line) and an additional dashed line defines the range of deviation less than a specified factor, which can be adjusted between 2 and 10 by the user. A second option allows for graphical display of

the ratio of predicted and measured concentrations per sampling site (Figure 8.3b). This graph shows the range of measured concentrations and gives an idea of the accuracy and bias of predicted concentrations. Data in both graphs can be displayed on linear and log axis, respectively. For stochastic simulation results, the user can select the mean or the median of predicted concentrations to be displayed. If river flow for all sampling events is known, a graphical comparison based on predicted loads is also possible. Additionally, for the evaluation of distributed concentrations a graphical is provided, illustrated in. The user can either evaluate measured concentrations against a percentile range as shown in Figure 8.3c (80% percentile range) or alternatively represent predicted concentrations as box-whisker plots.

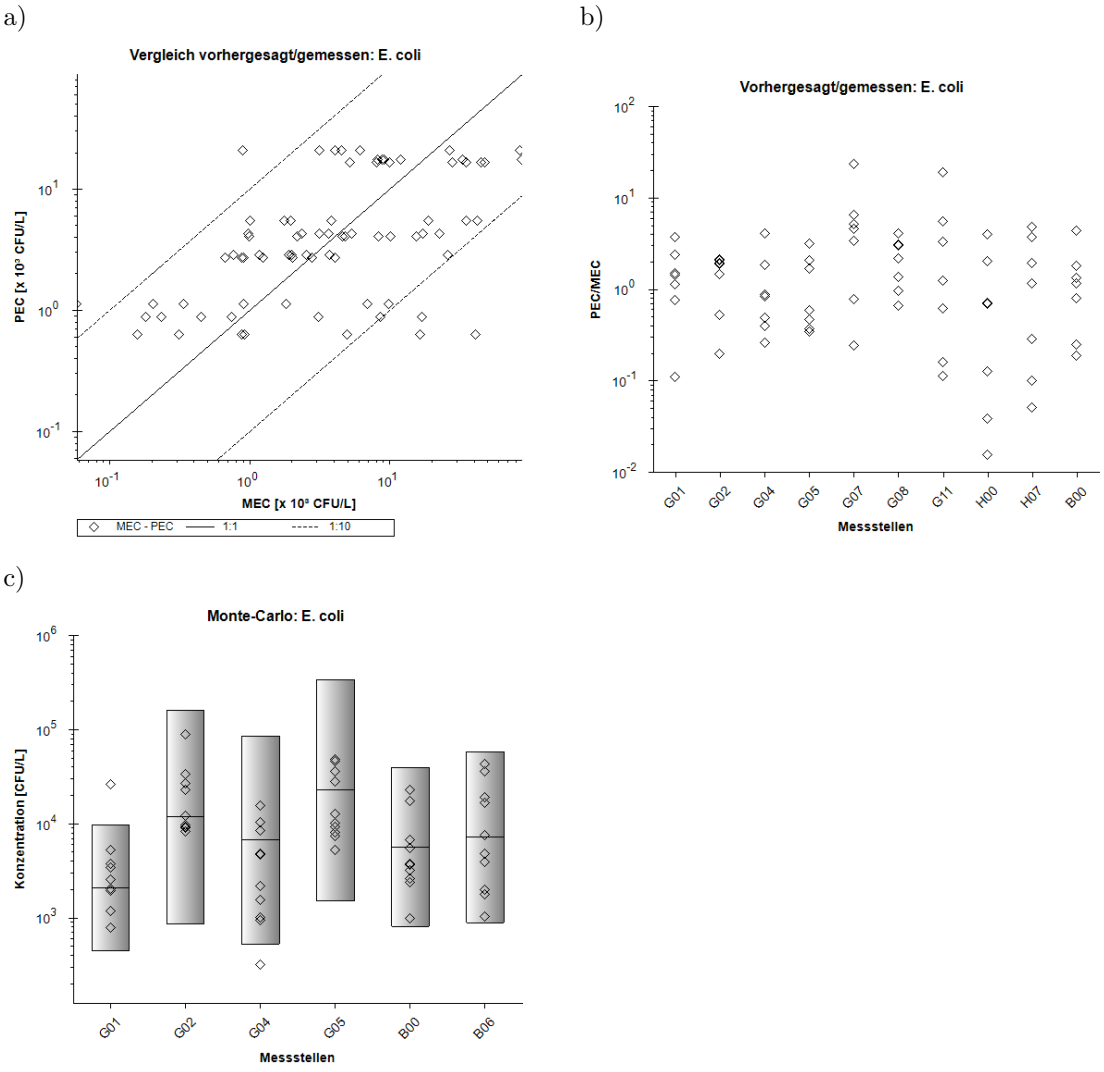


Figure 8.3: Graphical comparison of predicted and measured concentrations in the GREAT-ER software for deterministic simulations of all monitoring sites together (a) and separated by monitoring sites (b) as well as for Monte Carlo simulations (c). PEC: Predicted environmental concentrations, MEC: Measured environmental concentration.

8.4. Monte Carlo simulations

8.4.1. Overview

To account for natural variability and parameter uncertainty GREAT-ER offers the possibility to conduct simulations using a probabilistic Monte Carlo approach (Chapters 4 and 7). In this case, parameter values are described by probability distributions rather than deterministic values. In each individual simulation run (so-called MC shots), a random value is selected for distributed parameters according to the respective probability function. The simulation results are aggregated and stored as mean and standard deviation (Boeije et al., 1997). This is sufficient information under the assumption that the multiplicative model will result in lognormally distributed results independent of the individual parameter distributions (see below).

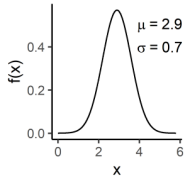
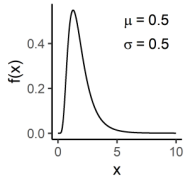
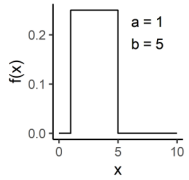
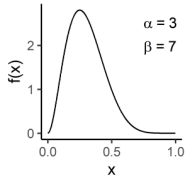
The number of Monte Carlo shots is usually between 10 000–100 000 for GREAT-ER simulations to achieve convergence; i.e. an approximately good estimation of the predicted concentrations. To make Monte Carlo simulations fully reproducible, the option to simulate with the very same parameter set was implemented. The user has to enter a so-called seed, which determines the numbers generated by the random number generator.

In the latest GREAT-ER 4.0 (Kehrein et al., 2015), a probabilistic description of flow rates was mandatory in Monte Carlo simulations. This made the evaluation of the impact of flow rates on the variance of predicted concentrations (Chapter 7) virtually impossible. This was enabled by adding the new option of constant flow rates in Monte Carlo simulations. In this case, flow rates are represented by the median flow rate.

8.4.2. Standard probability distribution functions

In GREAT-ER 4.0, three probability distributions were available for parameter description: Normal distribution, lognormal distribution and uniform distribution (see Table 8.2). The simplest way to account for parameter uncertainty is the uniform distribution, which is used when no detailed information on the actual parameter value distribution is available. It is defined by an upper and a lower limit and returns values within this range. Since all values in this range have the same probability, this distribution is suitable for a rather small range of values, e.g. ± 10% around the mean value (see e.g. Kehrein et al., 2015).

Table 8.2: Probability distributions implemented in GREAT-ER.

Distribution	Normal distribution[a]	Lognormal distribution[a]	Uniform distribution	Beta distribution[b]
Parameters	$\mu \in \mathbb{R}$: mean $\sigma^2 > 0$: variance	$\mu \in (-\infty, +\infty)$: mean of underlying normal distribution $\sigma > 0$: standard deviation of underlying normal distribution	$-\infty < a < b + \infty$ a: lower boundary, b: upper boundary	$\alpha > 0$: shape parameter $\beta > 0$: shape parameter
Probability density function f(x)	$\frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$ 	$\frac{1}{x\sigma\sqrt{2\pi}} \exp\left(-\frac{(\ln x-\mu)^2}{2\sigma^2}\right)$ 	$\begin{cases} \frac{1}{b-a} & \text{for } x \in [a,b] \\ 0 & \text{otherwise} \end{cases}$ 	$\frac{x^{\alpha-1}(1-x)^{\beta-1}}{B(\alpha,\beta)}$, where $B(\alpha,\beta) = \frac{\Gamma(\alpha)\Gamma(\beta)}{\Gamma(\alpha+\beta)}$ 
Cumulative distribution function F(x)	$\frac{1}{2}\left[1 + \text{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right]$	$\frac{1}{2}\left[1 + \text{erf}\left(\frac{\ln x-\mu}{\sigma\sqrt{2}}\right)\right]$	$\begin{cases} 0 & \text{for } x < a \\ \frac{x-a}{b-a} & \text{for } x \in [a,b] \\ 1 & \text{for } x > b \end{cases}$	$\frac{B(x;\alpha,\beta)}{B(\alpha,\beta)} = I_x(\alpha,\beta)$
Quantile $F^{-1}(p)$	$\mu + \sigma\sqrt{2}\text{erf}^{-1}(2p-1)$	$\exp(\mu + \sigma\sqrt{2}\text{erf}^{-1}(2p-1))$	$p(a+b)$	$I_p^{-1}(\alpha,\beta)$
Properties	$f(x)$ is symmetric around μ, $f(x)$ is bound by $-\infty$ and ∞	Supports only $x > 0$; i.e. $F^{-1}(p) > 0$	x is bound by a and b, all events in this range are equally likely	x is bound by 0 and 1, $f(x)$ is symmetric about 0.5 for $\alpha = \beta$
Potential utilization in GREAT-ER	Log reduction of bacterial by WWTP treatment (Chapter 7)	Flow rate (Chapters 4 and 7)	WWTP removal efficiency, surface photolysis rate (Kehrein et al., 2015)	Fraction of adsorbed bacteria (Chapter 7)

[a] erf is the error function and erf^{-1} is the inverse error function.

[b] $B(\alpha,\beta)$ is the beta function, Γ is the Gamma function, $B(x;\alpha,\beta)$ is the incomplete beta function, $I_x(\alpha,\beta)$ is the regularized incomplete beta function and $I_p^{-1}(\alpha,\beta)$ is the inverse regularized incomplete beta function.

Normally distributed observations can be found in various scientific disciplines, e.g. finance, ecology, or medicine. The normal distribution follows from the Central Limit Theorem: When a phenomenon arises from the additive interaction of many independent factors, the phenomenon typically approaches a normal distribution (Frank, 2009). The normal distribution is defined by its mean (μ) and variance (σ^2). The probability density function (PDF) is symmetric around the mean and unlimited.

Many phenomena in the environment are well represented by lognormally distributed variables, e.g. river flow rates. Thus, a basic assumption in the GREAT-ER model is that river flow at a specified site obeys to a lognormal distribution over time (Boeije et al., 1997). A variable is lognormally distributed when the natural logarithm of the observations follows a normal distribution. Lognormality emerges from the multiplicative interaction of different independent variables according to the Multiplicative Central Limit Theorem. Taking the logarithm of the product of many randomly distributed variables, one obtains the sum of randomly distributed variables, for which the Central Limit Theorem applies (Andersson, 2021). A lognormal distribution is defined by the mean and the standard deviation of the underlying normal distribution, i.e. mean and standard deviation of the log-transformed data. In contrast, in the GREAT-ER model the lognormal distribution is defined by the mean μ_X and standard deviation σ_X of the raw data and not the log-transformed data. Parameters of the underlying normal distribution are internally calculated according to

$$\mu = \ln\left(\frac{\mu_X^2}{\sqrt{\mu_X^2 + \sigma_X^2}}\right) \tag{8.2}$$

$$\sigma^2 = \ln\left(1 + \frac{\sigma_X^2}{\mu_X^2}\right). \tag{8.3}$$

8.4.3. Newly added probability distribution features

In this thesis, the set of available probability distributions was extended by the beta distribution and a modified normal distribution. The beta distribution probability distribution is commonly used for the representation of fractions as random variables, since it is limited to the range $[0, 1]$. In the context of environmental fate modeling, fractions often play a role, e.g. when calculating excreted loads of pharmaceuticals, removal fractions of environmental pollutants during WWTP treatment or the fraction of bacteria associated to suspended particles (Chapter 7).

The practical implementation of a probability distribution requires methods to generate deviates and to calculate cumulative probabilities and quantiles, i.e. the inverse cumulative distribution function. Beta distribution deviates are generated according to the BA

algorithm by Cheng (1978) and implemented according to McCaffrey (2019). The cumulative distribution function of the beta distribution is defined by the regularized incomplete beta function (Table 8.2). This is approximated by implementing the algorithm of van Winkle (2017) which utilizes Lentz's algorithm to evaluate continuous fractions Lentz (1982). Quantiles are calculated by implementing the algorithm by Cran et al. (1977) and subsequent remarks (Berry et al., 1991; Berry and Mielke, 1990). The computer code was adopted from the implementation of the algorithm in the R software (Gupta, 2016). Calculation of quantiles and the regularized incomplete beta function require an approximation of the Gamma function. This was implemented using the Lanczos approximation (Lanczos, 1964). The parameterization of the beta distribution requires input of the shape parameters α and β (Table 8.2). These can be obtained from the mean μ and standard deviation σ of a respective data set:

$$\alpha = \mu \left(\frac{\mu(1-\mu)}{\sigma^2} - 1 \right) \tag{8.4}$$

$$\beta = \alpha \frac{1-\mu}{\mu} \tag{8.5}$$

The normal distribution is unlimited. Therefore, realizations of normal distributed variables can theoretically be in the range from $-\infty$ to ∞. Since negative values are outside the codomain of some parameters, a modified version of the normal distribution was implemented (Chapter 7). To ensure only positive realizations, the routine generates a new random value, if the random number is less than zero. To maintain symmetry, the corresponding upper quantile of the distribution is truncated likewise.

9. Synthesis, discussion and outlook

9.1. Summary of main results

The modern world is hard to imagine without pharmaceuticals. They help us to sustain high quality of life and increase life expectancy (aus der Beek et al., 2016; Kümmerer, 2010; Wright and Weinstein, 1998). The importance of medical development became particularly evident during the COVID-19 pandemic. Only one year after the World Health Organization declared it a pandemic (March 11, 2020), the first vaccine was (fully) authorized in the European Union (May 28, 2021) (Pfizer, 2021; WHO, 2020a, 2021c). At the time of writing and submission of this thesis, the battle against the pandemic is still ongoing. It has caused millions of deaths, but although many challenges remain - e.g. inadequate access to vaccines in developing countries, virus mutations, and long-term health effects - the consequences of the pandemic would have been much worse without modern medicine and modern treatment methods (Del Rio et al., 2020; European Centre for Disease Prevention and Control, 2022; Padma, 2021; WHO, 2020b).

However, the residuals of chemicals used in Covid19-tretament as well end up in surface waters and can therefore potentially affect the ecosystem. This work has highlighted that catchment modeling can help conduct risk assessment, identify hot spots, and investigate the effectiveness of interventions. The GREAT-ER model has been demonstrated as valuable tool to simulate and evaluate pharmaceutical concentrations on a catchment scale. This thesis documents how the software was improved and a reduced, but more accessible model version was provided.

The case study on the simulation of pharmaceuticals in the catchment of the Vecht River yielded predicted environmental concentrations (PECs) in the range of µg L^{-1} (metformin) to less than ng L^{-1} (e.g. EE2). Risk assessment was conducted by comparing PECs with predicted no effect concentrations (PNECs). PNEC exceedance was predicted for diclofenac, carbamazepine and EE2 and in parts for erythromycin. The case study in the Vecht catchment underlines the classification of pharmaceuticals as chemicals of emerging concern (CEC) according to the definition of Lee et al. (2021) (see also Chapter 1): Simulated pharmaceuticals are poorly regulated, e.g, they are not (anymore) part of the

Water Framework Directive (WFD), they are incompletely degraded in WWTPs, most of them are poorly degraded in the environment, and they are predicted to be present in the aquatic environment at concerning concentrations close to posing risk to aquatic species.

In addition to chemical contamination of surface waters, this work also addressed microbial contamination, more specifically fecal contamination by *E. coli* and antibiotic-resistant (AR) *E. coli*. For this purpose, the GREAT-ER model was adapted to simulate (AR) *E. coli*. In deterministic simulation runs, the adapted model was found to reproduce well mean measured concentrations of *E. coli* and ESBL *E. coli* for average conditions. In contrast, the range of measured *E. coli* concentrations over the year was overestimated when the model was parametrized for Monte Carlo simulations, which can thus be considered conservative. Parameterization of emission sources (WWTPs and nonpoint sources) were shown to be primarily responsible for the variability in predicted concentrations. Concerning the loss processes, the sedimentation rate of dissolved particles had the greatest influence on the ranges of predicted concentrations. In deterministic scenarios, WWTPs were the primary emitters of *E. coli* into surface waters ($> 70\%$), especially for AR *E. coli* ($> 95\%$).

Exposure assessment of fecal pollution in the Vecht catchment, represented by *E. coli* concentrations, showed that under static conditions (deterministic simulations) swimming in the Vecht catchment is possible in many places. However, it also showed that limit concentrations are exceeded not only directly downstream of WWTPs, but can extend over a flow distance of several kilometers. The simulation of AR *E. coli* showed that during a single swimming event up to 30 CFU ESBL-EC and less than one CFU CP-EC can be taken up by ingestion. In the studied sub-catchment of the Vecht River, model predictions indicate that year-round swimming is not advisable in almost all surface waters impacted by wastewater.

Parts of the results obtained in this work have been incorporated into the MEDUWA Watershed Information System (WIS). This provides the possibility for the interested public to get information about the water quality in the Vecht catchment. On the one hand, this can promote the creation of awareness, on the other hand, the effectiveness of mitigation measures can be illustrated.

Various studies have shown that the GREAT-ER model is applicable to different catchments (see Section 1.1). The extension of the GREAT-ER software now allows for the opportunity to transfer the conducted studies to other catchments.

9.2. Methodological reflections and limitations

9.2.1. Study limitations

The GREAT-ER model, basically assumes a catchment-wide homogeneous flow rate percentile for all river segments within a simulation run. The underlying reasoning is that a flow situation which prevails upstream will also occur downstream. For larger catchments, however, this approach might be limited by climatological differences and different flow situations in large catchments (Boeije et al., 1997). In the Vecht catchment, two static flow situations were utilized for simulations: mean flow and low flow. These two scenarios represent average situations. For risk assessment of pharmaceuticals, they are suitable for comparison with threshold values targeting chronic toxicity (Chapters 4 and 5). For acute toxicity, Monte Carlo simulations are better suited because they better represent the range of expected concentrations as compared to deterministic simulation runs. PNECs applied for risk assessment in this thesis have considered only ecotoxicological effects as an endpoint. Antibiotics additionally can affect the resistome in the environment; i.e. promote for antibiotic resistance selection (Section 2.2.1). Bengtsson-Palme and Larsson (2016) calculated PNECs for antibiotic resistance selection. For the macrolide antibiotics clarithromycin and erythromycin considered in this work, no risk is found in this regard in the study areas examined. However, mixture effects are not included in the PNECs calculated by Bengtsson-Palme and Larsson (2016). The PNEC for resistance selection of ciprofloxacin (0.064 ng L^{-1}), is exceeded at several locations in the Vecht River (see Chapter 5), which is reason for concern. Another endpoint represents the human toxicological effects of pharmaceuticals; for example, via drinking water or unintentional ingestion of contaminated waters while swimming. This endpoint was not considered in the risk assessment.

When swimming in fecally contaminated water, potential infections from ingestion of these waters can be considered as an acute phenomenon. Therefore, Monte Carlo simulations might be better suited for estimating risk over the course of a year (Chapter 7) compared to static scenarios (Chapter 6). The chronical uptake of resistant bacteria through regular swimming and potential effects on the human body can be well-examined by the evaluation of static scenarios (Chapter 6). There is still a lack of knowledge about the effect of ingesting antibiotic-resistant bacteria (Leonard et al., 2018).

No input from agriculture was considered for the simulation of pharmaceuticals. However, the antibiotic erythromycin, for example, is also used as a veterinary antibiotic. Wöhler et al. (2020) estimated for the top 5 of veterinary antibiotics (excluding erythromycin) administered in Germany and the Netherlands that less than 100 g of the active substances

reach surface waters in the Vecht catchment over the course of a year. In contrast, approximately 70 kg of erythromycin were estimated to reach the surface waters in the catchment area via WWTP emissions calculated in Chapter 5. Therefore, it seems unlikely that erythromycin from the veterinary sector contributes significantly to pollution in the Vecht catchment. For a more profound statement, the fate of erythromycin from veterinary medicine could be estimated according to the approach of Wöhler et al. (2020).

To achieve the goals of this thesis, temporal variability was partially implemented in the GREAT-ER model. By modeling static hydrological scenarios with different boundary conditions (Chapters 5 and 6), it is possible in GREAT-ER to create and evaluate specific simulation environments, e.g. in terms of runoff, temperature or seasonality. Additional temporal variability can be simulated in stochastic simulations via correlation with the flow situation; e.g. for diffuse inputs and WWTP removal efficiency. Nevertheless, non-constant, i.e. time-variable, emissions remain the Achilles' heel in GREAT-ER. For the consideration of diffuse input, knowledge about loads on washed-off fields during a runoff event is necessary (Stoob et al., 2007). Furthermore, the validity of the steady state assumption during extreme weather events is not always given. The same applies to the resuspension of bacteria in sediment, since sediment concentrations can change over the course of the year (Kim et al., 2010) and remobilization of suspended material can also be event-driven (Pachepsky and Shelton, 2011).

9.2.2. Modeling versus monitoring

In this thesis, predicted concentrations of pharmaceuticals and *E. coli* were compared with measured concentrations. Both, modeling and monitoring complement each other and help to provide a more comprehensive picture of surface water pollution. A surface water sample depicts the situation, i.e. the respective concentration in a waterbody, at a specific point in time and space. In contrast, catchment-wide monitoring is virtually impossible. Additionally, with an increasing spatio-temporal resolution of measurement data, costs for equipment, laboratory and staff rise. In some cases, measurements are not functional, e.g. if the target substance or organism is present at very low concentrations or even below the detection limit (see e.g. Chapters 5 and 6).

Models always represent an abstract version of reality. The environment is a complex and highly variable system. Therefore, environmental models are often subject to inherent uncertainty. It is thus particularly advisable to (additionally) monitor vulnerable areas such as drinking water protection areas, conservation areas or bathing waters. Furthermore, measurement data are inevitable in systems where there is a lack of system knowledge or no understanding of the system at all. Here, monitoring data are the only basis to

conduct an environmental risk assessment. Additionally, the data can help to create a system understanding and to build and parameterize models (see Chapters 6 and 7). Finally, measurement data can be utilized for model evaluation and validation. By the comparison of predicted and measured values, the predictive power of models and their limitations can be evaluated (see. e.g. Chapters 5, 6).

9.3. Ideas for future research

9.3.1. Exposure modeling of pharmaceuticals and bacteria

Modeling enables to investigate future scenarios; e.g. the impact of climate change or social changes on water quality. Climate change affects the frequency, magnitude, and duration of hydrological events (Markovic et al., 2017). Water scarcity and droughts decrease dilution capacity (Sjerps et al., 2017), and heavy rainfall events increase the likelihood of CSO events and the intensity of surface runoff (Nilsen et al., 2011). Bunke et al. (2019) summarize socio-demographic trends that affect exposure to emerging pollutants in the environment. These include demographic change, global population growth, urbanization, and development in technologies. By simulating future scenarios, possible counter and mitigation measures can be evaluated years before potential consequences of climate change or societal developments occur. For example, based on the existing model for the Vecht catchment, scenarios for the prediction of future situations can be developed.

In this work, the fate of *E. coli*, ESBL-EC and CP-EC was predicted. For a more comprehensive assessment according to the EU Bathing Water Directive the other indicator bacteria (intestinal enterococci) could also be investigated. In addition to recreational activities in surface waters, there are other endpoints where humans are directly or indirectly affected by fecally contaminated waters; for example, during crop irrigation (Kokkinos et al., 2017) or drinking water abstraction (Khan et al., 2018). In potentially vulnerable areas, e.g. due to insufficient wastewater treatment efficiency, simulated bacterial concentrations can serve as a basis for risk assessment. In addition to the investigated AR bacteria, exposure to AR bacteria which are primarily prevalent in hospitals such as vancomycin-resistant enterococci (VRE) (Rossolini et al., 2014) could be performed. However, this would first require estimating VRE loads in hospital wastewater based on a proxy such as inpatient numbers, bed numbers, or specific departments.

9.3.2. GREAT-ER model perspectives

The simulation of a chemical parent compound could be extended in GREAT-ER by simulating the fate, transformation and re-transformation of the respective transformation products simultaneously. This requires a more detailed knowledge of transformation and degradation rates of the major degradation products of the selected substance. Sulfamethoxazole, for example, forms transformation products during metabolization and conventional wastewater treatment which can be back-transformed in the sewer, during the treatment process or in-stream (Bonvin et al., 2013; Jelic et al., 2015; Radke et al., 2009). With regard to transformation products, metformin should also be investigated more thoroughly. Its major degradation product guanylurea has a higher environmental toxic effect than the parent compound (Caldwell et al., 2019).

The *E. coli* model developed in this work could be further refined. For example, a more refined representation of diffuse emissions could provide more insight into the contribution of different sources in agricultural areas. An explicit consideration of bacteria introduced via horizontal transport requires a distinction of different flow components in GREAT-ER, i.e. baseflow, interflow, and surface runoff. In addition, the bacterial concentrations in the sediment and the load on the washed-off surfaces would need to be known. The approach of Kim et al. (2010) and Park et al. (2017), who distinguish active and passive transport of bacteria into the water column, can also be evaluated for inclusion.

The simulation of (AR) *E. coli* showed that the GREAT-ER model is not only applicable to classical down-the-drain chemicals. In recent years, microplastics have received increasing attention as environmental contaminants (Nizzetto et al., 2016). Major point sources of microplastics to surface waters are WWTPs, which retain 99% of microplastic loads from domestic and industrial wastewater, but still release substantial loads into the environment (Karbalaei et al., 2018). In addition, microplastics adsorbed in sewage sludge re-enter the environment via sewage sludge application to fields or storage in landfills. From there, they can be washed into adjacent surface waters (Nizzetto et al., 2016). Other diffuse sources include plastic products used in agriculture, such as plastic mulch, silage and fumigation films as well as anti-bird, or road wash-off, which flushes fragments of road markings or tire wear into adjacent surface waters (Karbalaei et al., 2018). Integrating a microplastic simulation routine could help identify spatially resolved important emission sources and help to generate a deeper understanding of the importance of different pathways.

In addition, the feasibility of a temporal component in the GREAT-ER model could be checked. Time has so far been included implicitly in form of temporal variability in flow rates or, in the bacteria model, through variability in temperature in form of Monte Carlo

simulations. For simulations of water quality in the Vecht catchment, another temporal component was introduced by simulating two distinct scenarios. It would be interesting to investigate to what extent a temporally resolved simulation, for example in monthly intervals, is reasonable considering the steady state assumption.

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A. Appendix to Chapter 4

Table A.1: Substance properties for model simulations.

		Clarithromycin	Iopamidol	Ethinylestradiol	Reference		
		I	II	III	I	II	III
Phys. chem. properties	Unit						
Molar mass	g/mol	747.96	777.08	296.1	[24]	[21]	[11]
log Kow		3.16	-2.42	3.67	[24]	[21]	[11]
Water solubility	mg/L	0.336	120	11.3	[24]	[21]	[26]
pKa		8.95	10.7	10.4	[24]	[21]	[26]
WWTP removal							
Lagoon	%	30.5	< 10	> 70	[20]	[10]	[8]
Wetland	%	30.5	< 10	> 70	[20]	[10]	[8]
Biofilm	%	44	60–80	86	-	[6]	[4]
Activated Sludge	%	44	35	87	[9], [19], [24]	[10]	[4], [15]
River removal							
Half-life	d		> 44		-	[17]	-
Model assumption	1/h		6.6×10^{-4}		-	[23]	-
Near surface photolysis	1/h	0.001	-	5.5×10^{-3}	[20]	-	[16]
Kd river	L/kg	335	-	140	[1]	-	[16]
Consumption							
Per capita consumption	kg/(cap yr)	1.28×10^{-4}	6.6×10^{-4}	5.596×10^{-7}	[22]	[13]	[27]
Hospital fraction	%	15.2	87.5	-	-	[7]	-
Excretion	%	30	87.5	40	[2], [19]	[7]	[14]

[1] Azuma et al. (2017a), [2] Baumann et al. (2015), [3] Carvalho et al. (2015), [4] Clara et al. (2004), [5] Coutu et al. (2013), [6] Escolà Casas et al. (2015), [7] Duchin et al. (1986), [8] Froehner et al. (2011), [9] Göbel et al. (2005), [10] Götz et al. (2012), [11] Hansch et al. (1995), [12] Hijosa-Valsero et al. (2011), [13] IKSR) (2010), [14] Johnson and Williams (2004), [15] Joss et al. (2004), [16] Jürgens et al. (2002), [17] Kormos et al. (2010), [18] Kormos et al. (2011), [19] Kümmerer and Henninger (2003), [20] Nakada et al. (2007), [21] O'Neil (2006), [22] Schwabe et al. (2017), [23] Ternes and Hirsch (2000), [24] Ternes et al. (2007), [25] Vione et al. (2009), [26] Yalkowsky and Yan. (2003), [27] Intercontinental Medical Statistics (IMS Health) (2014).

Table A.2: Main characteristics of investigated river basins.

	Unit	Main	Lenne	Naab
Size	[km^2]	27 250	1 352	5 225
Connected inhabitants		$\sim$ 3 800 000	$\sim$ 380 000	$\sim$ 500 000
Number of WWTPs		848	36	192
Flow length of the main stream	[km]	527	129	98
Cumulated length of the simulated river network	[km]	10 273	5 156	2 077
MQ-discharge at the outlet point	[m^3/s]	$\sim$ 250	$\sim$ 28	$\sim$ 50

B. Appendix to Chapter 5

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B.1 STP emission estimation

Table B.1: Pharmaceutical consumption rates.

Compound	German per capita consumption Vecht catchment [kg/(cap yr)] [a]	Dutch per capita consumption Vecht catchment [kg/(cap yr)] [b]	Ratio German to Dutch per bed consumption [c]
17α-Ethinylestradiol	1.50×10^{-7}	6.39×10^{-7}	n.a. [d]
Carbamazepine	4.36×10^{-4}	4.56×10^{-4}	203%
Ciprofloxacin	2.75×10^{-4}	2.32×10^{-4}	131%
Cyclophosphamide [e]	1.93×10^{-6}	0	46%
Diclofenac	6.73×10^{-4}	2.54×10^{-4}	198%
Erythromycin	3.14×10^{-4}	1.98×10^{-5}	117%
Metformin	1.36×10^{-2}	1.97×10^{-2}	144%
Metoprolol	1.47×10^{-3}	1.74×10^{-3}	123%

[a] IQVIA Commercial GmbH & Co. OHG, calculations based on IMS PharmaScope® (2018).

[b] Dutch Foundation for Pharmaceutical Statistics (2018).

[c] Annual per bed consumption rates were calculated as the mass of prescribed pharmaceuticals in a hospital devided by the number of beds in the respective hospital. These values were averaged for German and Dutch hospitals, respectively. Due to the limited number of hospitals which provided data and data security issues only ratios of the average per bed consumption ratios can be displayed. Excluding ethinylestradiol and cyclophosphamide, the per-bed consumption rate is 1 to 40 times higher than the per capita consumption rates of the respective countries.

[d] In both countries, no hospital consumption data was reported; n.a., not applicable.

[e] Cyclophosphamide is restricted to clinical use in the Netherlands. SFK only collects domestic pharmaceutical consumption. Therefore, no domestic cyclophosphamide use is recorded in for the Netherlands.

Table B.2: Pharmaceutical excretion data. Urinary and faecal excretion percentages. Glucuronide conjugates of the parent compound are shown in brackets a. For the modelling exercise (sixth column), mean urinary excretion and 20% of mean faecal excretion were applied.

Compound	Urine (+ conjugates) [%]	Faeces (+ conjugates) [%]	Urine + faeces (+ conjugates) [%]	Source	Modelled fraction entering STPs [%]
17α-Ethinylestradiol	10.1 (17.2)	23.1		12	32
Carbamazepine	< 10	< 30		1	15
	0.8 (11)	13 (?)		2	
	2	< 28		3	
	1.44	12.3		4	
			2.7–15 (0)	5	
	1	28		6	
			1–2 (30)	7	
			31	8	
Ciprofloxacin	40–50	< 20–35		1	54
	< 70	15		1	
	44.7	25		3	
	61.5	15.2		3	
Cyclophosphamide	25	"small amounts"		1	25
Diclofenac	? (< 15)	< 5		1	10%
	2–23 [b]	1–4 [b]		9	
			1 (10–15)	5	
			0.05–0.1 (0.5–1.5)	5	
	6	< 35		6	
			2 (15)	8	
			15 (< 1)	7	
			< 1 (5–10)	10	
Erythromycin	4–20	40–50		11	19
			4	8	

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Table B.2 – continued from previous page

Compound	Urine (+ conjugates) [%]	Faeces (+ conjugates) [%]	Urine + faeces (+ conjugates) [%]	Source	Modelled fraction entering STPs [%]
	5–10	"large amounts"		1	
	5	"mainly"		3	
	12–15	"mainly"		3	
Metformin	30–50	30		1	74
	35–50	30		13	
	79	0		13	
	100	0		14	
	100	0		15	
Metoprolol	< 10	-		1	8
	< 5	-		3	
	3–10			16	
	9.4	-		17	
	< 5.2	-		17	
			7	8	
			3–10	7	
	5–10	-		10	

1. Moffat et al. (2011). 2. Bahlmann et al. (2014). 3. Swiss Agency for Therapeutic Products (2020). 4. Björlenius et al. (2018). 5. Heberer and Feldmann (2005). 6. Zhang et al. (2008). 7. Ternes and Joss (2008). 8. Khan and Ongerth (2004). 9. Johnson et al. (2007). 10. Kümmerer et al. (2011). 11. Göbel et al. (2005). 12. Johnson and Williams (2004). 13. Tucker et al. (1981). 14. Robert et al. (2003). 15. Bristol-Myers Squibb (2018). 16. Alder et al. (2010). 17. Regårdh et al. (1974).

[a] Glucuronide conjugates can react back to the parent compound in the sewer (Gao et al., 2017; Heberer and Feldmann, 2005; Kumar et al., 2012). For this study, we assume that the entire fraction excreted as glucuronide associated parent compound will react back to the parent compound in the sewer. Therefore, we aggregate the excretion rates of the parent compound and the glucuronide conjugates of the parent compound in a single excretion rate.

[b] No distinction between parent compound and conjugates.

Except for diclofenac and erythromycin, all compounds were applied systematically solely. For the latter STP inflow loads (L_{in} [kg/yr]) were calculated as

$$L_{in} = pCC \times Inh \times f_{ex} \tag{B.1}$$

where pCC is the per capita consumption rate [kg/(cap yr)], Inh [cap] is the number of inhabitants in the STP catchment and f_{ex} is the fraction that is excreted in the unchanged or conjugated state.

Only the absorbed portion of topically applied erythromycin and diclofenac are thought to undergo metabolism. Sioufi et al. (1994) found relative proportions of parent compounds and metabolites after topical application compared to oral application for diclofenac. The portion that is not absorbed either goes into clothing, bandages or is wiped off with e.g. paper and then thrown in the trash Heberer and Feldmann (2005). According to Heberer and Feldmann (2005), STP inflow loads of diclofenac can be estimated as

$$L_{in} = (f_{sys} \times f_{ex} + f_{top} \times f_{ab} \times f_{ex} + f_{top} \times (1 - f_{ab})) \times pCC \times Inh \tag{B.2}$$

where f_{sys} is the systematically applied fraction, f_{top} is the topically applied fraction and f_{ab} the fraction that is absorbed after topical application. For the scope of this study we use worst case estimations and assume that the fraction which is not absorbed $(1 - f_{ab})$ ends up in the wastewater, e.g. via washing of clothing or bandages. In the model of Heberer and Feldmann (2005) it is assumed that 100% of the parenterally or orally administered dose is absorbed leading to the same excretion rate regardless of the route of administration. This model is also used to calculate the influent loads of erythromycin. Parameters for Germany and the Netherlands are shown in Table B.3. The result for diclofenac is that in Germany 52% and in the Netherlands 15% of the total prescribed mass ends up in wastewater. For erythromycin this results in 21% and 31% for Germany and the Netherlands, respectively.

Table B.3: Diclofenac and erythromycin inflow model parameters.

	Germany [a]		Netherlands [b]			
Compound	f_{sys}	f_{top}	f_{sys}	f_{top}	f_{ab}	f_{ex} [e]
Diclofenac	0.51	0.49	0.97	0.03	0.07 [c]	0.10
Erythromycin	0.99	0.01	0.86	0.14	0.00 [d]	0.19

[a] IQVIA Commercial GmbH & Co. OHG, calculations based on IMS PharmaScope® (2018).
[b] Dutch Foundation for Pharmaceutical Statistics (2018).
[c] Hui et al. (1998).
[d] Systematically exposure of topically applied erythromycin is negligible (Carls et al., 2014).
[e] Table B.2.

Table B.4: Summary of removal efficiencies published in literature. Removal efficiencies have to be interpreted as percentage change of mass loading in effluent versus influent. Negative removal efficiencies may occur when the back-reaction of labile intermediates to the parent compound outweigh the actual removal or due to experimental and analytical uncertainty for compounds with low removal efficiencies (< 10%). STP, sewage treatment plant; SD, standard deviation.

Compound	Number of STPs	Mean [%]	SD [%]	Median [%]	Sources
17α-Ethinylestradiol	3	72.5	5.5	70.5	3, 18
Carbamazepine	33	-5.8	27.5	0.0	1, 3, 4, 7, 8, 10, 11, 12, 13, 15, 18, 20, 21
Ciprofloxacin	22	71.1	20.1	78.0	1, 4, 6, 9, 21
Cyclophosphamide	1	59.0	0.0	59.0	2
Diclofenac	19	25.5	22.7	31.2	3, 4, 8, 11, 12, 13, 15, 17, 18, 19, 20
Erythromycin	21	14.0	29.7	14.6	1, 4, 5, 6, 8, 9, 10, 12, 13, 14, 16, 18
Metformin	6	97.4	1.2	97.5	4, 11, 15
Metoprolol	16	22.1	27.6	22.9	4, 7, 8, 11, 12, 13, 15, 18, 19, 21, 22

1. Castiglioni et al. (2006). 2. Česen et al. (2015). 3. Clara et al. (2005). 4. de Jesus Gaffney et al. (2017). 5. Göbel et al. (2007). 6. Guerra et al. (2014). 7. Gurke et al. (2015). 8 Kasprzyk-Hordern et al. (2009). 9. Li and Zhang (2011). 10. Nakada et al. (2007). 11. Oosterhuis et al. (2013). 12. Radjenovic et al. (2007). 13 Radjenović et al. (2009). 14. Roberts and Thomas (2006). 15. Sacher (2014). 16. Senta et al. (2019). 17. Sui et al. (2011). 18. Ternes et al. (2007). 19. Thomas et al. (2007). 20. Vergeynst et al. (2015). 21. Vieno et al. (2006). 22. Wick et al. (2009).

Table B.5: Parameterization of in-stream processes. CBZ, carbamazepine; CIP, ciprofloxacin; CYL, cyclophosphamide; DFC, diclofenac; ERY, erythromycin; EE2, 17α-ethinylestradiol; MET, metformin; MEP, metoprolol.

Compound	Surface photolysis rates a [1/h]	Source	First order degradation rate [a] [1/h]	Source	Bio degradation rate [1/h]	Source	Kd [b] [L/kg]	Source
CBZ	Scn_{AC}: 1.1×10^{-4} Scn_{DS}: 2.2×10^{-4}	Estimated with a quantum yield of 1.1×10^{-5} Calisto et al. (2011)			$< 1 \times 10^{-4}$	Durán-Álvarez et al. (2015)	13.3 [d]	Radović et al. (2016)
CIP	Scn_{AC}: 0.647 Scn_{DS}: 1.311	Estimated with an average quantum yield of 8.5×10^{-3} (at pH 7.5)			No degradation	Girardi et al. (2011)	250	Tolls (2001)
CYC			7×10^{-4}	Buerge et al. (2006)	$< 1 \times 10^{-4}$	Lutterbeck et al. (2016)	4.4 [e]	Azuma et al. (2017a)
DFC	Scn_{AC}: 0.018 Scn_{DS}: 0.049	Estimated with a quantum yield of 0.038 Andreozzi et al. (2003)			Recalcitrant	Lahti and Oikari (2011)	14.4 [d]	Radović et al. (2016)

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Compound	Surface photolysis rates a [1/h]	Source	First order degradation rate a [1/h]	Source	Bio degradation rate [1/h]	Source	Kd [b] [L/kg]	Source
ERY			0.003	Batchu et al. (2014)	$< 1 \times 10^{-4}$	Alexy et al. (2004)	139.7 [d]	Radović et al. (2016)
EE2	0.0029 [c]	Jürgens et al. (2002)			Resistant to biodegradation	Zuo et al. (2013)	278	Ternes et al. (2004)
MET			0.0012	Neamţu et al. (2014)	Not readily biodegradable	Trautwein and Kümmerer (2011)	19	Scheurer et al. (2009)
MEP			0	Baena-Nogueras et al. (2017)	0.001	Baena-Nogueras et al. (2017)	18.1 [d]	Radović et al. (2016)

[a] Seasonal surface photolysis rates were estimated based on wavelength-dependent sunlight intensities at 50 degree north latitude Apell and McNeill (2019) and available light absorption spectra of the substance. Quantum yields were taken from the literature. No quantum yields and no seasonal photolysis rates were available for ethinylestradiol. Therefore, we applied the same literature photolysis rate for both scenarios. Cyclophosphamide, erythromycin, metformin and metoprolol do not effectively absorb sunlight in the photochemically relevant wavelength range between 295 nm–400 nm. For these compounds, lumped pseudo first order degradation rates reported in the literature were used without correction for seasonal influences due to a lack of more detailed information.

[b] The distribution coefficient Kd is an input parameter of the GREAT-ER model to estimate the chemical fraction of a chemical prone to sedimentation in a river segment. Sedimentation is modelled using the equilibrium distribution assumption represented by an average Kd value between suspended matter and water. The model includes a basic assumption on the average suspended matter concentration which is used to estimate the adsorbed fraction. Spatial information about the composition and properties of suspended matter in the Vecht River catchment was not available. Furthermore, information about Kd values between suspended matter and water in natural rivers was not available for the investigated APIs. However, Kd values are reported in the literature for the sediment-water, soil-water and sludge-water equilibrium. Therefore, those values were used as a proxy to describe the suspended matter water distribution in the Vecht River catchment.

[c] Based on assumed 12 hours of sunlight exposure per day.

[d] Average value of four sediments.

[e] Average value of two sediments.

B.2 Monitoring campaign

As a part of a one-year sampling campaign of bacteria and bacteria resistance genes in the Vecht catchment (omitted author, in preparation) a subset of collected STP and in-stream samples was analysed for pharmaceuticals (Tables B.6–B.8). The selection of STPs was the same as in the grand sampling campaign. Selection was based on the plant location (Germany/Netherlands), the plant scale (small to large) and, if the plant was or was not treating hospital wastewater. Approximately 50% of STP influent and effluent samples were analysed for pharmaceuticals. This was thought to be sufficient to cover pharmaceutical variability in STP influent and effluent in Germany and the Netherlands. The sampling months of the STP measurements are displayed in Table B.7. For two STPs a gradient measurement was performed, i.e. Hardenberg and Steinfurt-Burgsteinfurt. For these plants, one surface water sample upstream of each plant was taken (sampling sites H00 and S00 respectively), as well as several surface water samples downstream of the plants (sampling sites H01–H06 and S02–S06 respectively). Furthermore, several surface water locations were sampled for other interests. One sample was taken on the location where the river crosses the German-Dutch border (sampling site G11). The other sampling sites were distributed across the catchment (sampling sites G02, G04, G05, G07, G08, G09, G10). The in-stream sampling sites represent a subset of the sampling sites in the grand monitoring campaign and were taken on locations that were important for evaluation of the GREAT-ER model. At each of these sampling sites a fraction of samples was analysed for pharmaceuticals. These fractions were selected based on the date of sampling and the hydrological conditions on the respective day. At Dutch sampling sites pumping activities were also taken into account. The in-stream sampling sites and their allocation to the scenarios are summarized in Table B.8.

Table B.6: Number of the sampling sites and number of samples taken in the Vecht catchment. For a comprehensive overview see Chapter 6 with Appendix C. STP, sewage treatment plant.

	STP (influent and effluent)	In-stream
Germany	Gronau, Nordhorn, Schüttorf, Steinfurt-Burgsteinfurt ($n_{influent}$ = 25, $n_{effluent}$ = 25)	S00, S02, S03, S04, S05, S06, G02, G04, G05 (n_{ScnDS} = 18, n_{ScnAC} = 28)
Netherlands	Almelo-Sumpel, Dalfsen, Enschede-West, Hardenberg, Ootmarsum, Vroomshoop ($n_{influent}$ = 34, $n_{effluent}$ = 33)	H00, H02, H03, H04, H06, G06, G07, G09, G10, G11($nScn_{DS}$ = 19, $nScn_{AC}$ = 27)

Table B.7: Sampling dates of the subset of samples and allocation to scenarios.

Sampling month and year	STP [a]
July 2018	W01, W02, W04, W05, W07, W09, W10, W11
August 2018	W01, W02, W04, W05, W07, W09, W10, W11
November 2018	W01, W02, W04, W05, W07, W09, W10, W11
December 2018	W03, W06
January 2019	W03, W04, W06, W07
February 2019	W01, W02, W03, W04, W05, W07, W09, W10
March 2019	W03, W04, W05, W06, W07, W09
April 2019	W01, W02, W03, W04, W05, W06, W07, W10, W11
May 2019	W02, W03, W06, W09, W11

[a] W01, Hardenberg. W02, Enschede. W03, Steinfurt-Burgsteinfurt. W04, Nordhorn. W05, Ootmarsum. W06, Gronau. W07, Schuettorf. W09, Almelo-Sumpel. W10, Dalfsen. W11, Vroomshoop.

Table B.8: Sampling dates of the subset of samples and allocation to scenarios. Scn_{DS}, dry summer scenario; Scn_{AC}, average condition scenario.

Allocated scenario	Sampling month and year	Sampling sites
Scn_{DS}	June 2018	S00, S02, S03, S04, S05, S06, G02, G04, G05, G07, G08, G09, G10, G11, H00, H02, H03, H04, H06
Scn_{DS}	August 2018	S00, S03, S04, S05, S06, G02, G04, G05, G07, G08, G09, G10, G11, H00, H03, H04, H06
Scn_{DS}	September 2018	S02
Scn_{AC}	October 2018	S00, S02
Scn_{AC}	November 2018	S00, S02, S03, S04, S05, S06, G02, G04, G05, G07, G08, G09, G10, G11, H00, H03, H04, H06
Scn_{AC}	December 2018	S00, S01, S03, S04, S05, S06
Scn_{AC}	February 2019	S00
Scn_{AC}	March 2019	S00, G05, G07, G08, G09, G10, G11, H00, H03, H04, H06
Scn_{AC}	April 2019	S00, S03, S04, S05, S06, G02, G04, G05, G07, G08, G09, G10, H00, H03, H04, H06
Scn_{AC}	May 2019	S00, G11

Determination of micropollutants

Water samples were taken and stored at -20 °C within 6 hours of collection. For sample preparation 2000 µL of thawed sample was mixed with 200 µL of methanol and 100 µL of modifier solution, shaken for 30 minutes at high speed using a Heidolph shaker. After centrifugation, 900 µL of supernatant was pipetted into LC-MS vials. 13C standard addition was carried out in all samples and results have been corrected accordingly. The analysis was conducted using a Agilent 6420 Triple Quadrupole LC-MS/MS system with an electrospray ion source. A thorough description of the analysis has been reported elsewhere (omitted author unpublished manuscript). For this study, four compounds have been added to the method later. The mass/charge per compound and recovery rates are listed in Table B.9 and Table B.10, respectively.

Table B.9: Mass/charge per compound.

Compound	Precursor ion	Product ion	Retention time (min)	Fragmentor voltage (V)	Collision energy (V)	Polarity
Metoprolol	268.2	191	4.75	125	15	Positive
Metoprolol	268.2	116	4.75	125	16	Positive
Carbamazepine	237.2	194.2	5.83	155	16	Positive
Carbamazepine	237.2	179.1	5.83	155	40	Positive
Naproxen	231	185	6.48	90	10	Positive
Naproxen	231	170	6.48	90	28	Positive
Diclofenac	296	215	7.22	95	17	Positive
Diclofenac	296	214	7.22	85	32	Positive

Table B.10: Compound recovery rates. Recoveries for the measured compounds varied between 70–136%. Recoveries were determined individually for each samples to cancel any variations do to the water matrix.

Compound	Mean recovery (standard deviation) [%]
Carbamazepine	91.50 (27.19)
Ciprofloxacin	77.52 (32.60)
Diclofenac	136.00 (34.04)
Erythromycin	85.93 (30.55)
Metformin	77.60 (29.35)
Metoprolol	90.88 (27.04)

B.3 Baseline for 'benchmarking'

To provide a reliable baseline for the 'benchmarking' approach, predicted carbamazepine concentrations (C_{pred} [ng/L]) were first evaluated by comparison with measured concentrations (C_{meas} [ng/L]). To make predicted and measured concentrations comparable, concentration data from monitoring sites where daily flow rates (Q_{meas} [m^3/d]) were available were adjusted (C_{adj} [ng/L]) to the flow rate used in the model simulation (Q_{model} [m^3/d]),

$$C_{adj} = C_{meas} \times Q_{meas}/Q_{model} \tag{B.3}$$

B.4 Aquatic ecotoxicity data

Table B.11: Literature studies retrieved from Web of Science Core Collection ('Topic' search mode).

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NO	Aaen, S. M., & Horsberg, T. E. (2016). A screening of multiple classes of pharmaceutical compounds for effect on preadult salmon lice Lepeophtheirus salmonis. Journal of Fish Diseases, 39(10), 1213-1223. doi:10.1111/jfd.12463
YES	Aderemi, A. O., Novais, S. C., Lemos, M. F. L., Alves, L. M., Hunter, C., & Pahl, O. (2018). Oxidative stress responses and cellular energy allocation changes in microalgae following exposure to widely used human antibiotics. Aquatic Toxicology, 203, 130-139. doi:10.1016/j.aquatox.2018.08.008
NO	Affek, K., Zaleska-Radziwill, M., Doskocz, N., & Debek, K. (2018). Mixture toxicity of pharmaceuticals present in wastewater to aquatic organisms. Desalination and Water Treatment, 117, 15-20. doi:10.5004/dwt.2018.21964
NO	Ajima, M. N. O., Pandey, P. K., Kumar, K., & Poojary, N. (2017). Neurotoxic effects, molecular responses and oxidative stress biomarkers in Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) exposed to verapamil. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, 196, 44-52. doi:10.1016/j.cbpc.2017.03.009
NO	Alfei, S., Catena, S., Ponassi, M., Rosano, C., Zoppi, V., & Spallarossa, A. (2018). Hydrophilic and amphiphilic water-soluble dendrimer prodrugs suitable for parenteral administration of a non-soluble non-nucleoside HIV-1 reverse transcriptase inhibitor thiocarbamate derivative. European Journal of Pharmaceutical Sciences, 124, 153-164. doi:10.1016/j.ejps.2018.08.036
NO	Alimba, C. G., Adekoya, K. O., & Soyinka, O. O. (2019). Exposure to effluent from pharmaceutical industry induced cytogenotoxicity, hematological and histo-pathological alterations in clarias gariepinus (Burchell, 1822). Excli Journal, 18, 63-78. doi:10.17179/excli2018-1916
NO	Almeida, A. R., Jesus, F., Henriques, J. F., Andrade, T. S., Barreto, A., Koba, O., . . . Domingues, I. (2019). The role of humic acids on gemfibrozil toxicity to zebrafish embryos. Chemosphere, 220, 556-564. doi:10.1016/j.chemosphere.2018.12.133
NO	Al-Saeedi, A. H., Al-Ghafri, M. T. H., & Hossain, M. A. (2017). Brine shrimp toxicity of various polarities leaves and fruits crude fractions Ziziphus jujuba native to Oman and their antimicrobial potency. Sustainable Chemistry and Pharmacy, 5, 122-126. doi:10.1016/j.scp.2017.03.003

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NO	Ashajyothi, C., Handral, H. K., & Kelmani, R. C. (2018). A Comparative In Vivo Scrutiny of Biosynthesized Copper and Zinc Oxide Nanoparticles by Intraperitoneal and Intravenous Administration Routes in Rats. Nanoscale Research Letters, 13. doi:10.1186/s11671-018-2497-2
NO	Backhaus, T. (2016). Environmental Risk Assessment of Pharmaceutical Mixtures: Demands, Gaps, and Possible Bridges. Aaps Journal, 18(4), 804-813. doi:10.1208/s12248-016-9907-0
YES	Baek, I. H., Kim, Y., Baik, S., & Kim, J. (2019). Investigation of the Synergistic Toxicity of Binary Mixtures of Pesticides and Pharmaceuticals on Aliivibrio fischeri in Major River Basins in South Korea. International Journal of Environmental Research and Public Health, 16(2). doi:10.3390/ijerph16020208
NO	Balkrishna, A., Sharma, N., Sharma, V. K., Mishra, N. D., & Joshi, C. S. (2018). Green synthesis, characterisation and biological studies of AgNPs prepared using Shivlingi (Bryonia laciniosa) seed extract. Iet Nanobiotechnology, 12(3), 371-375. doi:10.1049/iet-nbt.2017.0099
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NO	Benelli, G., Govindarajan, M., AlSalhi, M. S., Devanesan, S., & Maggi, F. (2018). High toxicity of camphene and gamma-elemene from Wedelia prostrata essential oil against larvae of Spodoptera litura (Lepidoptera: Noctuidae). Environmental Science and Pollution Research, 25(11), 10383-10391. doi:10.1007/s11356-017-9490-7

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YES	Bi, R., Zeng, X. F., Mu, L., Hou, L. P., Liu, W. H., Li, P., . . . Xie, L. T. (2018). Sensitivities of seven algal species to triclosan, fluoxetine and their mixtures. Scientific Reports, 8. doi:10.1038/s41598-018-33785-1
YES	Bialk-Bielinska, A., Mulkiewicz, E., Stokowski, M., Stolte, S., & Stepnowski, P. (2017). Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery-Biological effects and stability under test conditions. Chemosphere, 189, 689-698. doi:10.1016/j.chemosphere.2017.08.174
YES	Bittner, L., Teixido, E., Seiwert, B., Escher, B. I., & Kluver, N. (2018). Influence of pH on the uptake and toxicity of beta-blockers in embryos of zebrafish, Danio rerio. Aquatic Toxicology, 201, 129-137. doi:10.1016/j.aquatox.2018.05.020
YES	Bohdziewicz, J., Dudziak, M., Kaminska, G., & Kudlek, E. (2016). Chromatographic determination and toxicological potential evaluation of selected micropollutants in aquatic environment-analytical problems. Desalination and Water Treatment, 57(3), 1361-1369. doi:10.1080/19443994.2015.1017325
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YES	Caldwell, D. J., D'Aco, V., Davidson, T., Kappler, K., Murray-Smith, R. J., Owen, S. F., . . . Tell, J. (2019). Environmental risk assessment of metformin and its transformation product guanylurea: II. Occurrence in surface waters of Europe and the United States and derivation of predicted no-effect concentrations. Chemosphere, 216, 855-865. doi:10.1016/j.chemosphere.2018.10.038
YES	Capolupo, M., Diaz-Garduno, B., & Martin-Diaz, M. L. (2018). The impact of propranolol, 17-ethinylestradiol, and gemfibrozil on early life stages of marine organisms: effects and risk assessment. Environmental Science and Pollution Research, 25(32), 32196-32209. doi:10.1007/s11356-018-3185-6
NO	Cartagena, A. F., Esmerino, L. A., Polak, R., Parreiras, S. O., Michel, M. D., Farago, P. V., & Campanha, N. H. (2017). New denture adhesive containing miconazole nitrate polymeric microparticles: Antifungal, adhesive force and toxicity properties. Dental Materials, 33(2), E53-E61. doi:10.1016/j.dental.2016.09.039
NO	Carty, D. R., Thornton, C., Gledhill, J. H., & Willett, K. L. (2018). Developmental Effects of Cannabidiol and Delta(9)-Tetrahydrocannabinol in Zebrafish. Toxicological Sciences, 162(1), 137-145. doi:10.1093/toxsci/kfx232
YES	Cesen, M., Elersek, T., Novak, M., Zegura, B., Kosjek, T., Filipic, M., & Heath, E. (2016). Ecotoxicity and genotoxicity of cyclophosphamide, ifosfamide, their metabolites/transformation products and their mixtures. Environmental Pollution, 210, 192-201. doi:10.1016/j.envpol.2015.12.017
YES	Chen, H. H., Gu, X. H., Zeng, Q. F., & Mao, Z. G. (2019). Acute and Chronic Toxicity of Carbamazepine on the Release of Chitobiase, Molting, and Reproduction in Daphnia similis. International Journal of Environmental Research and Public Health, 16(2). doi:10.3390/ijerph16020209
YES	Chiffre, A., Clerandeau, C., Dwoinikoff, C., Le Bihanic, F., Budzinski, H., Geret, F., & Cachot, J. (2016). Psychotropic drugs in mixture alter swimming behaviour of Japanese medaka (Oryzias latipes) larvae above environmental concentrations. Environmental Science and Pollution Research, 23(6), 4964-4977. doi:10.1007/s11356-014-3477-4

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NO	Cil, O., Phuan, P. W., Lee, S., Tan, J., Haggie, P. M., Levin, M. H., . . . Verkman, A. S. (2016). CFTR Activator Increases Intestinal Fluid Secretion and Normalizes Stool Output in a Mouse Model of Constipation. Cellular and Molecular Gastroenterology and Hepatology, 2(3), 317-327. doi:10.1016/j.jcmgh.2015.12.010
NO	Clausen, L. P. W., & Trapp, S. (2017). Toxicity of 56 substances to trees. Environmental Science and Pollution Research, 24(22), 18035-18047. doi:10.1007/s11356-017-9398-2
NO	Cui, F., Chai, T. T., Qian, L., & Wang, C. J. (2017). Effects of three diamides (chlorantraniliprole, cyantraniliprole and flubendiamide) on life history, embryonic development and oxidative stress biomarkers of Daphnia magna. Chemosphere, 169, 107-116. doi:10.1016/j.chemosphere.2016.11.073
NO	Cunha, D. L., Mendes, M. P., & Marques, M. (2019). Environmental risk assessment of psychoactive drugs in the aquatic environment. Environmental Science and Pollution Research, 26(1), 78-90. doi:10.1007/s11356-018-3556-z
YES	Czarny, K., Szczukocki, D., Krawczyk, B., Skrzypek, S., Miekos, E., & Gadzala-Kopciuch, R. (2019). Inhibition of growth of Anabaena variabilis population by single and mixed steroid hormones. Journal of Applied Phycology, 31(1), 389-398. doi:10.1007/s10811-018-1589-9
NO	da Silva, L. D., Gozzi, F., Sires, I., Brillas, E., de Oliveira, S. C., & Machulek, A. (2018). Degradation of 4-aminoantipyrine by electro-oxidation with a boron-doped diamond anode: Optimization by central composite design, oxidation products and toxicity. Science of the Total Environment, 631-632, 1079-1088. doi:10.1016/j.scitotenv.2018.03.092
NO	Dalhoff, K., Gottardi, M., Kretschmann, A., & Cedergreen, N. (2016). What causes the difference in synergistic potentials of propiconazole and prochloraz toward pyrethroids in Daphnia magna? Aquatic Toxicology, 172, 95-102. doi:10.1016/j.aquatox.2015.12.007
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NO	Dawson, D. A., & Poch, G. (2017). Evaluation of consistency for multiple experiments of a single combination in the time-dependence mixture toxicity assay. Toxicology Mechanisms and Methods, 27(9), 707-716. doi:10.1080/15376516.2017.1351019
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YES	de Garcia, S. O., Garcia-Encina, P. A., & Irusta-Mata, R. (2016). Dose-response behavior of the bacterium Vibrio fischeri exposed to pharmaceuticals and personal care products. Ecotoxicology, 25(1), 141-162. doi:10.1007/s10646-015-1576-8
YES	de Oliveira, L. L. D., Nunes, B., Antunes, S. C., Campitelli-Ramos, R., & Rocha, O. (2018). Acute and Chronic Effects of Three Pharmaceutical Drugs on the Tropical Freshwater Cladoceran Ceriodaphnia silvestrii. Water Air and Soil Pollution, 229(4). doi:10.1007/s11270-018-3765-6
NO	Dechayont, B., Limpichai, C., Kornwisitwathin, K., Nuengchamnong, N., & Itharat, A. (2017). In vitro cytotoxic and antioxidant activities of Pikut Trichinthalamaga remedy. Oriental Pharmacy and Experimental Medicine, 17(3), 233-238. doi:10.1007/s13596-017-0278-6
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YES	Zivna, D., Plhalova, L., Chromcova, L., Blahova, J., Prokes, M., Skoric, M., . . . Svobodova, Z. (2016). The effects of ciprofloxacin on early life stages of common carp (Cyprinus carpio). Environmental Toxicology and Chemistry, 35(7), 1733-1740. doi:10.1002/etc.3317
NO	Zortea, T., dos Reis, T. R., Serafini, S., de Sousa, J. P., da Silva, A. S., & Baretta, D. (2018). Ecotoxicological effect of fipronil and its metabolites on Folsomia candida in tropical soils. Environmental Toxicology and Pharmacology, 62, 203-209. doi:10.1016/j.etap.2018.07.011
NO	Zuriaga, E., Lomba, L., German, B., Lanuza, P. M., Aldea, L., Ribate, M. P., . . . Giner, B. (2019). Ecotoxicity in Aliivibrio fischeri of Ibuprofen, Omeprazole and their Mixtures. Chemistry and Ecology, 35(2), 102-114. doi:10.1080/02757540.2018.1540608

Table B.12: CRED scores evaluating the reliability and relevance of critical literature articles for their inclusion in the derivation of safe concentration in this study.

Articles	Reliability	Relevance	Sufficient quality? [a]
Aderemi et al. (2018)	R1	C1	Yes
Ando et al. (2007)	R4	C2	No
Bayer et al. (2014)	R4	C4	No
Chen et al. (2019)	R1	C2	Yes
de Liguoro et al. (2009)	R2	C2	Yes
Di Poi et al. (2018)	R1	C2	Yes
Dordio et al. (2011)	R3	C2	No
Eguchi et al. (2004)	R4	C1	No
Fabbri et al. (2014)	R3	C4	No
Godoy et al. (2018)	R1	C1	Yes
González-Pleiter et al. (2013)	R2	C2	Yes
Han et al. (2006)	R3	C1	No
He et al. (2013)	R2	C2	Yes
Jarvis et al. (2014)	R2	C3	No
Ji et al. (2012)	R2	C1	Yes
Jungmann et al. (2017)	R2	C2	Yes
Li et al. (2010)	R2	C2	Yes
Majewska et al. (2018)	R2	C1	Yes
Martins et al. (2012)	R2	C2	Yes
Ofoegbu et al. (2019)	R3	C3	No
Russo et al. (2018)	R2	C1	Yes
Yang et al. (2008)	R3	C1	No
Yokota et al. (2018)	R1	C1	Yes
Załęska-Radziwiłł et al. (2011)	R4	C1	No
Zhu et al. (2014)	R2	C1	Yes
Zounková et al. (2007)	R4	C2	No

[a] Studies deemed of sufficient quality had to be assign reliability scores of R1 or R2, and relevance scores of C1 or C2.

Literature search string

The titles, abstracts, and keywords were screened using the following search string "(LC50* OR EC50* OR EC10* OR NOEC* OR "effect concentration") AND (aquatic* OR *water*) AND (*toxic*) AND (pharmaceutic* OR medicine* OR drug* OR ((amantadine OR *amant*) OR (carbamazepine OR carbamaz*) OR (ciprofloxacin OR ciproflox*) OR (cyclophosphamide OR c*clo*os*amid*) OR (diclofenac OR diclofenac*) OR (doxycycline OR dox*c*clin*) OR (erythromycin OR er*throm*cin*) OR (ethinylestradiol OR *ethinyl*estradiol) OR (iopamidol OR io*ami* OR "contrast agent") OR (metformin OR metformi* OR dimethylbiguanid* OR dimethylimidodicarbonimidic) OR (metoprolol OR "1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]-2-[4]propanol") OR (oxazepam OR "7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one") OR (phenazone OR phenazon* OR antipyrine OR "1,5-Dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one") OR (sul*amethazine OR sul*adimidin* OR sul*adimethylpyrimidine) OR (valsartan))) NOT QSAR". At the time of the search, additional compounds besides the eight pharmaceuticals of interest in this study were included, retrieving a total of 233 publications. All these publications were screened in detail but only the ones containing information on the eight pharmaceuticals of interest in this study were used.

Species names

Harmonized according to most recent taxonomic nomenclature and corrected for misspellings.

Exposure type

"Chronic" or "acute" classification was primarily assigned according to the authors. If not explicitly mentioned, a decision was made according to the corresponding original methods article referenced (if readily available), or (inter)national chemical testing guidelines (e.g. OECD Test No. 201). Alternatively, the life span of the organism and the exposure duration was considered. In this regard, a 10% lifespan coverage threshold was applied as to decide whether to classify an exposure as chronic or acute (Suter II et al., 2006). For example, Danio rerio lives on average 1 year in the wild; bioassays with exposure times higher than 10% of 365 days where tagged "chronic". Similarly, this threshold was applied in early development stage data under the assumption that exposure during this critical period can potentially exert long-term effects further in the lifecycle. If no exposure time, guideline or protocol were provided, the values were conservatively classified as "acute".

Effect code

If effects were not reported or unspecified, these were coded as "UND" (undetermined). Population effects reported as more than one effect like "Survival, reproduction and growth rate" were coded as "POP" (population). In the case of multiple effects in which one or more effects do not necessarily dictate the sustainability of a population, such as "Length, reproduction and survival", were attributed the code "MUL" (multiple).

Endpoints

When authors did not explicitly use LOEC or NOEC terminology, the publications, graphs were inspected to assign the corresponding concentration values according to the results of the statistical tests. In studies where single concentrations were tested, if effects were determined significant, that concentration was classified as "LOEC". If not significant, a "<" was assigned. Highest concentrations tested showing no effects tagged by the authors as "NOEC" and assigned with ">" were recorded. If not explicitly classified by the authors, these values were coercively assigned ">" to distinguish from studies where both NOEC and LOEC were derived empirically.

Exposure duration

If several exposure times were given (e.g. interval, 176–301 days) associated with only one effect value, the highest time point is used (e.g. sampled at 8–60 days, only 60 days is accounted for).

Concentration units

Given the intent of this assessment, only aquatic exposure measured in weight of test substance per volume (e.g. mg/L) were included. All concentrations were converted to µg/L. Unit conversion from molar to µg/L was done using the molecular weight (MW) provided by authors, chemical manufacture company, PubChem (https://pubchem.ncbi.nlm.nih.gov/) or other relevant source. The CAS numbers were used to extract MW. If CAS was not disclosed then the substance name and the corresponding best match result was used.

Substance aggregation

Different forms or variations of a parent substance were aggregated (Table B.13) to circumvent the scarcity of substance-specific effect data and pool compounds with analogous

biological activity (e.g. metoprolol tartrate and metoprolol succinate) or metabolically related (e.g. carbamazepine and carbamazepine metabolite trans-10,11-dihydroxy-10,11-dihydrocarbazepine). Moreover, this aggregation prevents overly stringent data exclusion due to incomplete identification of the substance (e.g. missing CAS registry number).

Table B.13: Grouping of pharmaceuticals.

Group	Compounds
Amantadine	amantadine
Carbamazepine	carbamazepine carbamazepine 10,11-epoxide trans-10,11-dihydroxy-10,11-dihydrocarbazepine
Ciprofloxacin	ciprofloxacin ciprofloxacin HCl
Cyclophosphamide	cyclophosphamide carboxycyclophosphamide keto-cyclophosphamide N-dechloroethyl-cyclophosphamide
Diclofenac	diclofenac diclofenac Na
Doxycycline	doxycycline
Erythromycin	erythromycin erythromycin phosphate
Ethinylestradiol	ethinylestradiol 17α-ethinylestradiol
Iopamidol	iopamidol
Metformin	metformin metformin HCl
Metoprolol	metoprolol metoprolol tartrate metoprolol succinate
Oxazepam	oxazepam
Phenazone	phenazone
Sulfamethazine	sulfamethazine sulfadimidine
Valsartan	valsartan

B.5 Predicted no-effect concentration

Endpoint Aggregation

The aggregation of endpoints was done following established guidelines (European Chemicals Agency, 2008) and according to their closest or equivalent toxicological effect response (Table B.14). Aggregated endpoints are in the present study referred simply as 'endpoints'.

Table B.14: Grouping of available endpoints in the database into aggregated chronic NOEC, chronic EC50, acute NOEC and acute EC50 endpoints. MATC was reverse calculated to obtain NOEC.

Chronic exposure		Acute exposure	
NOEC	EC50	NOEC	EC50
EC10	EC50	EC5	L(E)C50
EC5	ET50	EC10	EC20
IC10	IC50	LC10	EC25
IC5	LC50	MATC	EC50
LC01		NOEC	IC50
LC10		NOEL	LC50
MATC			MTC
NOAEC			
NOEC			
NOEL			

Table B.15: Chronic ecotoxicological effects on freshwater species. To derive predicted no-effect concentrations (PNEC) for each substance a distinct assessment factor (AF) was applied to the most sensitive species and effect depending on the data available.

Substance	Taxa	Species	Effect	Concentration (µg/L)	AF	PNEC (µg/L)
Carbamazepine	insecta	Stenonema sp.	BEH	0.2	10	0.02
	crustacea	Daphnia similis	REP	0.3		
	algae	Chaetophora sp.	POP	2		
	crustacea	Daphnia pulex	REP	100		
	fish	Pimephales promelas	BEH	100		
	insecta	Chironomus riparius	DEV	164		
	fish	Oncorhynchus mykiss	GRO	180		
	crustacea	Ceriodaphnia dubia	REP	199		
	rotifera	Brachionus calyciflorus	REP/ MOR	377		
	crustacea	Daphnia magna	REP/ GRO	400		
	crustacea	Hyalella azteca	MOR	600		
	algae	Chlorella pyrenoidosa	POP	1000		
	algae	Scenedesmus acutus	POP	1000		
	algae	Raphidocelis subcapitata	POP	2046		
	insecta	Chironomus tentans	GRO	2600		
	fish	Oryzias latipes	BEH	6150		

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Table B.15 – continued from previous page

Substance	Taxa	Species	Effect	Concentration (µg/L)	AF	PNEC (µg/L)
	algae	Cyclotella meneghiniana	POP	10000		
	algae	Chlorella vulgaris	POP	11800		
	fish	Danio rerio	REP	12500		
Ciprofloxacin	fish	Lebistes reticulatus	GRO	780	10	78
	fish	Poecilia reticulata	GRO	780		
	algae	Raphidocelis subcapitata	POP	3006		
	crustacea	Daphnia magna	REP	3217		
Cyclophosphamide	crustacea	Ceriodaphnia dubia	POP	1250	10	125
	rotifera	Brachionus calyciflorus	POP	3394		
	algae	Raphidocelis subcapitata	POP	12500		
	fish	Danio rerio	MOR	13743785		
Diclofenac	mollusca	Dreissena ploymorpha	MOR	0.5	50	0.01
	fish	Oryzias latipes	DEV	7.29		
	fish	Danio rerio	GRO	10		
	fish	Oncorhynchus mykiss	REP/ DEV/ MOR	1084		
	algae	Raphidocelis subcapitata	GRO	25000		

Continued on next page

Table B.15 – continued from previous page

Substance	Taxa	Species	Effect	Concentration (µg/L)	AF	PNEC (µg/L)
	algae	Chlamydomonas reinhardtii	POP	32700		
Erythromycin	cyanobacteria	Anabaena sp.	POP	5	10	0.5
	algae	Raphidocelis subcapitata	POP	23		
	crustacea	Daphnia magna	GRO	11100		
	crustacea	Moina macrocopa	MOR/ REP	50000		
	fish	Oryzias latipes	MOR	100000		
17α-Ethinylestradiol	fish	Gobiocypris rarus	REP	0.00018	50	3.6×10^{-6}
	fish	Danio rerio	DEV	0.00069		
	fish	Rutilus rutilus	GRO	0.00071		
	fish	Syngnathus scovelli	DEV	0.001		
	fish	Salmo trutta	GRO	0.00208		
	fish	Gasterosteus aculeatus	DEV	0.00418		
	amphibia	Lithobates septentrionalis	GRO/ DEV	0.005		
	amphibia	Lithobates clamitans	REP	0.0058		
	fish	Salvelinus namaycush	GRO	0.0063		
	fish	Pimephales promelas	GRO/ REP	0.008		
	fish	Oryzias latipes	REP	0.00669		

Continued on next page

Table B.15 – continued from previous page

Substance	Taxa	Species	Effect	Concentration (µg/L)	AF	PNEC (µg/L)
	mollusca	Bithynia tentaculata	GRO	0.009		
	mollusca	Radix balthica	GRO	0.009		
	fish	Cyprinodon variegatus	REP	0.009		
	fish	Alburnus tarichi	REP	0.01		
	amphibia	Xenopus tropicalis	DEV	0.0175		
	fish	Etheostoma caeruleum	DEV	0.02		
	mollusca	Potamopyrgus antipodarum	REP	0.025		
	fish	Syngnathus abaster	MOR	0.02655		
	fish	Poecilia reticulata	DEV/ POP	0.05		
	mollusca	Lymnaea stagnalis	DEV	0.05		
	fish	Oncorhynchus mykiss	REP	0.05965		
	fish	Fundulus heteroclitus	MOR	0.1		
	crustacea	Gammarus pulex	POP	0.1		
	mollusca	Haitia pomilia	POP	0.1		
	fish	Tautogolabrus adspersus	MOR	0.1		

Continued on next page

Table B.15 – continued from previous page

Substance	Taxa	Species	Effect	Concentration (µg/L)	AF	PNEC (µg/L)
	mollusca	Marisa cornuarietis	REP	0.5		
	crustacea	Daphnia magna	REP	14		
	crustacea	Sida crystallina	DEV	32		
	crustacea	Acartia tonsa	DEV	46		
	crustacea	Hyalella azteca	GRO/ MOR	70		
	insecta	Chironomus tentans	POP	88.32		
	crustacea	Ceriodaphnia reticulata	MOR	200		
	crustacea	Ceriodaphnia dubia	REP	500		
Metformin	crustacea	Daphnia similis	REP	4400	10	440
	crustacea	Ceriodaphnia dubia	REP	7900		
	fish	Pimephales promelas	DEV	10000		
	fish	Brachydanio rerio	DEV	11713		
	crustacea	Daphnia magna	REP/ MOR	26593.859		
	algae	Raphidocelis subcapitata	POP	99749		
	cnidarian	Hydra attenuata	REP	701800		
Metoprolol	crustacea	Daphnia magna	REP	3100	10	310
	algae	Raphidocelis subcapitata	POP	6786		

Continued on next page

Table B.15 – continued from previous page

Substance	Taxa	Species	Effect	Concentration (µg/L)	AF	PNEC (µg/L)
	crustacea	Gammarus fossarum	REP	15000		
	protozoa	Tetrahymena pyriformis	GRO	21800		
	fish	Danio rerio	GRO	24000		

Table B.16: Predicted no-effect concentration estimations from literature and this study. Bold numbers indicate values uniquely calculated in this study.

Substance	PNEC (µg/L)	References
17α-Ethinylestradiol	1.6×10^{-2}, 1×10^{-4}, 3.7×10^{-5}, 3.5×10^{-5}, 3.1×10^{-5}, 2×10^{-5}, 3.6×10^{-6}, 3×10^{-8}	1-9, 22
Carbamazepine	170, 130, 17, 10, 8, 2.6, 2.5, 2, 0.5, 0.4, 0.05, **0.02**	1, 3, 5, 8-20
Ciprofloxacin	**78**, 0.5, 0.45, 0.089, 0.005	3, 5, 6, 9, 15, 21, 22
Cyclophosphamide	1120, 980, 560, **125**	9, 13, 22
Diclofenac	50, 32, 31, 20, 10, 0.45, 0.1, 0.05, 0.02, **0.01**	2, 3, 5, 6, 7, 8, 9, 13, 15, 18, 20, 22, 24, 23
Erythromycin	2, 0.5, **0.3**, 0.2	3, 5, 6, 8, 9, 21
Metformin	1030, 1000, 780, 640, **440**, 156, 100, 20, 13.45, 10, 4.2	3, 5, 7, 8, 9, 12, 14, 16, 17, 25-27
Metoprolol	**310**, 75, 64, 62, 58.3, 31, 8.6, 7.3, 3.2	3, 5, 8, 9, 12, 16, 17, 22, 28-30

1. van Vlaardingen et al. (2007). 2 van der Aa et al. (2011). 3. Oekotoxzentrum (2016). 4. Oekotoxzentrum (2016). 5. NORMAN-network (2020). 6. Loos et al. (2018). 7. Vestel et al. (2016). 8. Ågerstrand and Rudén (2010). 9. Perazzolo et al. (2010). 10. Triebskorn et al. (2007). 11. Heye et al. (2019). 12. Lif et al. (2019). 13. Boxall et al. (2014). 14. Comber et al. (2018). 15. Frédéric and Yves (2014). 16. Moermond (2014). 17. Moermond et al. (2016). 18. Ferrari et al. (2004). 19. Wenzel and Shemotyuk (2014). 20. Gheorghe et al. (2016). 21. AMR Industry Alliance (2020). 22. Grung et al. (2008). 23. Hoeger et al. (2005). 24. European Union (2011). 25. Caldwell et al. (2019). 26. Oekotoxzentrum (2016). 27. AstraZeneca (2017a). 28. Oekotoxzentrum (2016). 29. AstraZeneca (2017b). 30. Murray-Smith et al. (2012).

B.6 Model evaluation

Emission estimation

Five APIs (carbamazepine, ciprofloxacin, diclofenac, metformin and metoprolol) had a quantification frequency above 90% in STP influent and were included in the model evaluation exercise. Figure 5.2 shows that the majority of the predicted influent loads (> 85%) agree within a factor of 3 with loads derived from measured concentrations. Except for two outliers in the Netherlands, all data points were within a factor of 10 indicating an acceptable overall model performance (Figure B.1). Country-specific evaluation reveals differences; influent loads show a small overestimation in Germany and the Netherlands ($SSPB_{GER}$ = 17%, $SSPB_{NL}$ = 6%). Erythromycin showed a quantification frequency of less than 50% in both, German and Dutch STPs. Even though, when erythromycin concentrations below the LOQ were replaced by the LOQ value, i.e. the highest possible quantifiable concentration, influent loads for erythromycin in German STPs are highly overestimated by the model (SSPB = 296%). Since all other processes (excretion patterns, in-sewer processes) were assumed equal in German and Dutch Vecht regions, erroneous German consumption volumes were most likely responsible for this bias. To bring the overestimation to an acceptable level German erythromycin consumption was adjusted by a factor of 0.5 (SSPB = 99%) as to account for unknown influencing factors.

In the next step, predicted effluent loads were compared to data (Figure 5.3 and Figure B.2). After STP removal, four APIs (carbamazepine, diclofenac, metformin and metoprolol) had a quantification frequency above 90%. Overall, predictions of STP effluent loads agreed well with empirical data, showing good accuracy ($\xi_{effluent}$ = 64%) and small underestimation ($SSPB_{effluent}$ = −22%). Ciprofloxacin loads were very largely overestimated (SSPB = 288%) even when measured concentrations below the LOQ were replaced with the LOQ (Figure B.2). Adjusting ciprofloxacin emissions by a factor of 0.5 lead to an acceptable bias (SSPB = −94%).

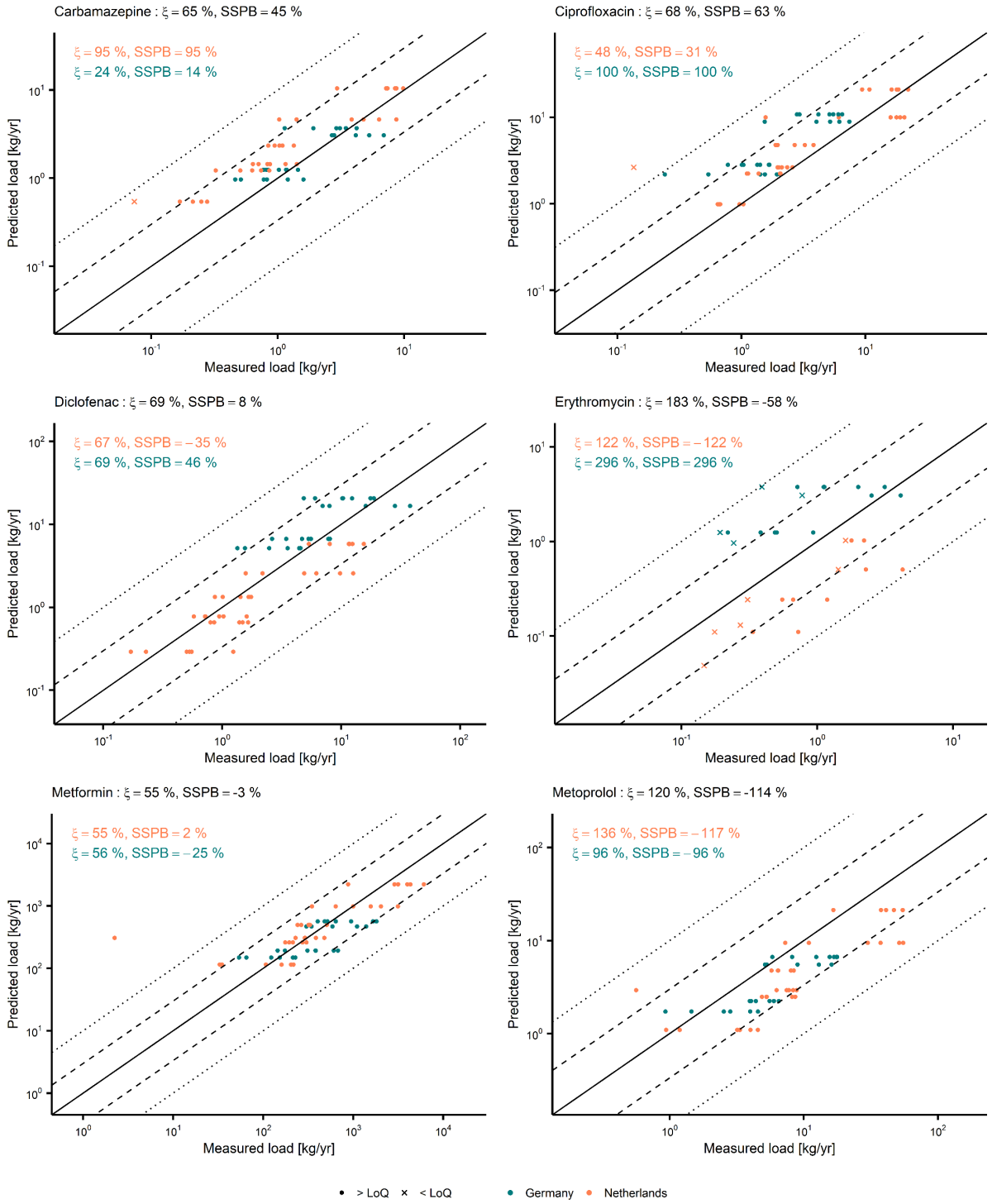

Figure B.1: Predicted and measured STP influent loads of APIs with a detection frequency above 25%. Dashed lines indicate the 1:3 and 3:1 ratios, dotted lines indicate the 1:10 and 10:1 ratios. All APIs were measured 25 times in German and 34 times in Dutch STPs. Concentrations below the LOQ are processed as LOQ. Actual concentrations are therefore lower and measures (ξ, SSPB) should be taken with care for substances with many concentrations below the LOQ, i.e. erythromycin.

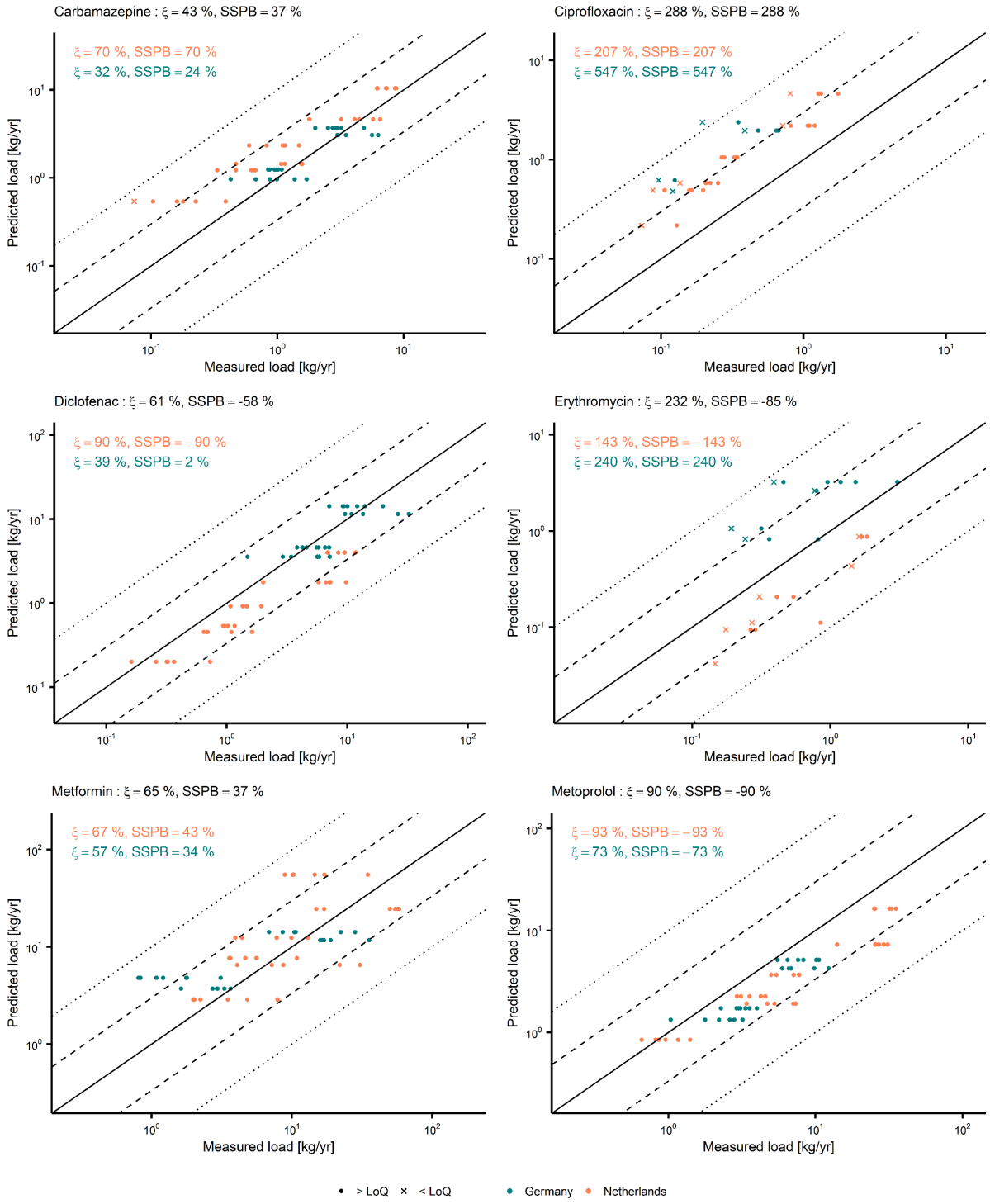

Figure B.2: Predicted and measured STP effluent loads of APIs with a detection frequency above 25%. Dashed lines indicate the 1:3 and 3:1 ratios, dotted lines indicate the 1:10 and 10:1 ratios. All APIs were measured 25 times in German and 33 times in Dutch STPs. Concentrations below the LOQ are processed as LOQ. Actual concentrations are therefore lower and measures (ξ, SSPB) should be taken with care for substances with many concentrations below the LOQ, i.e. ciprofloxacin and erythromycin.

In-stream evaluation

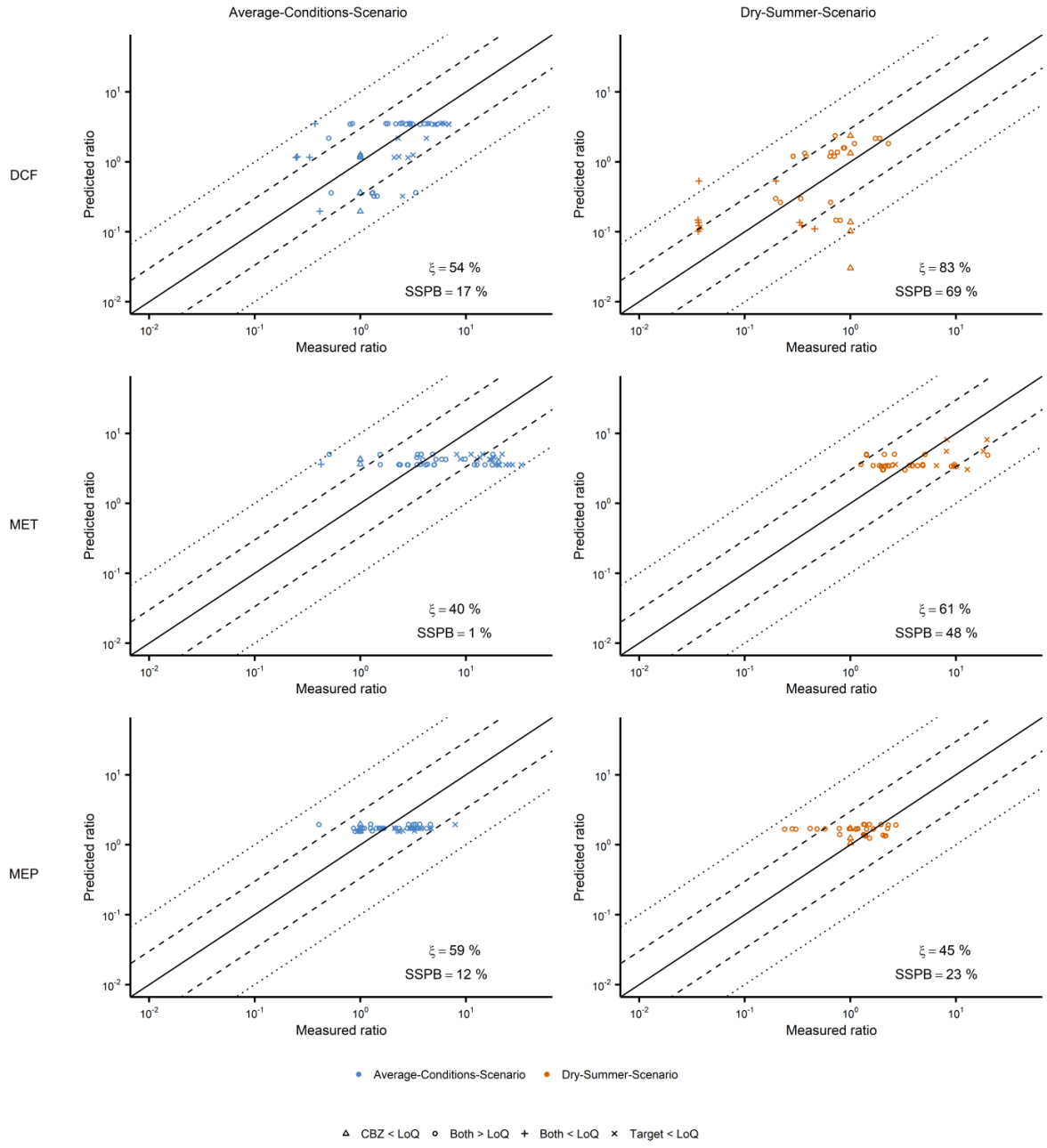

Figure B.3: Predicted and measured benchmark ratios of diclofenac, metformin and metoprolol at monitoring sites in the whole Vecht catchment. Dashed lines indicate the 1:3 and 3:1 ratios, dotted lines indicate the 1:10 and 10:1 ratios. Measures were calculated including predicted-measured pairs where both, the target compound and carbamazepine concentrations, were above the LOQ.

B.7 Risk assessment

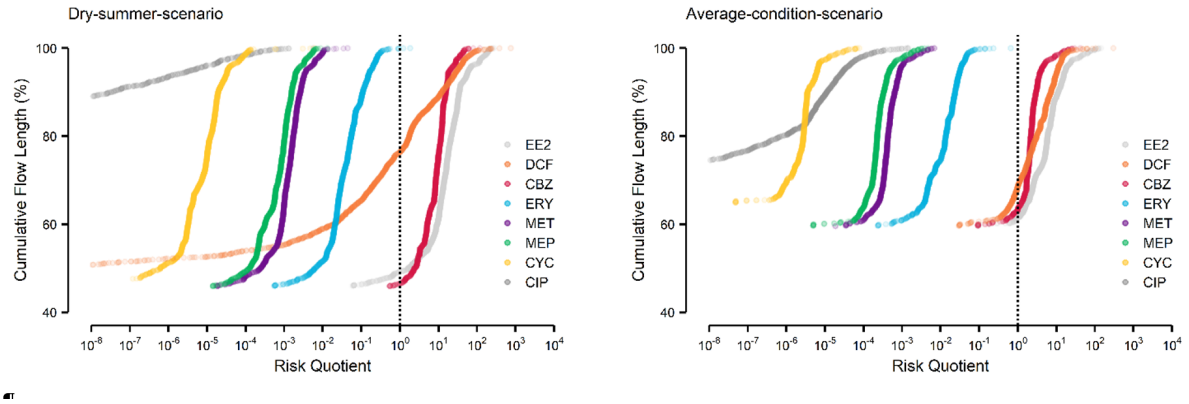

Figure B.4: Percentage of the Vecht catchment flow length at risk of environmental pharmaceutical pollution. Vertical black dashed line indicates the safe threshold RQ = 1, i.e. predicted environmental concentrations equal to the predicted no-chronic-effect concentration. Risk quotients below 10-8 are not depicted in this figure. The Y-axis minimum value was set to 40% since more than 40% of the flow length have RQ = 0. Each point depicts a water stream segment of ≤ 2 km. CBZ, carbamazepine; CIP, ciprofloxacin; CYL, cyclophosphamide; DFC, diclofenac; ERY, erythromycin; EE2, 17α-ethinylestradiol; MET, metformin; MEP, metoprolol.

Table B.17: Water volume percentage and flow length percentage of the Vecht River catchment vulnerable to different ranges of active pharmaceutical ingredients (API) risk quotients (RQ). CBZ, carbamazepine; CIP, ciprofloxacin; CYL, cyclophosphamide; DFC, diclofenac; ERY, erythromycin; EE2, 17α-ethinylestradiol; MET, metformin; MEP, metoprolol.

		Average-condition-scenario					Dry-summer-scenario				
	API	[0, 0]	(0, 0.1]	(0.1, 1]	(1, 10]	(10, +∞)	[0, 0]	(0, 0.1]	(0.1, 1]	(1, 10]	(10, +∞)
Water volume [%]	EE2	9			65	25	2		1	9	87
	CBZ	9		1	89		2			66	32
	CIP	9	91				3	97			
	CYC	12	88				2	98			
	DCF	9		23	63	4	2	37	34	23	3
	ERY	9	91				2	91	7		
	MET	9	91				2	98			
	MEP	9	91				2	98			
Flow length [%]	EE2	59		1	27	11	46		3	12	39
	CBZ	59		3	35	2	46		1	29	24
	CIP	59	40				48	52			
	CYC	65	35				48	52			
	DCF	59	1	8	26	6	46	19	11	12	11
	ERY	59	40				46	43	11		
	MET	59	40				46	54			
	MEP	59	40				46	54			

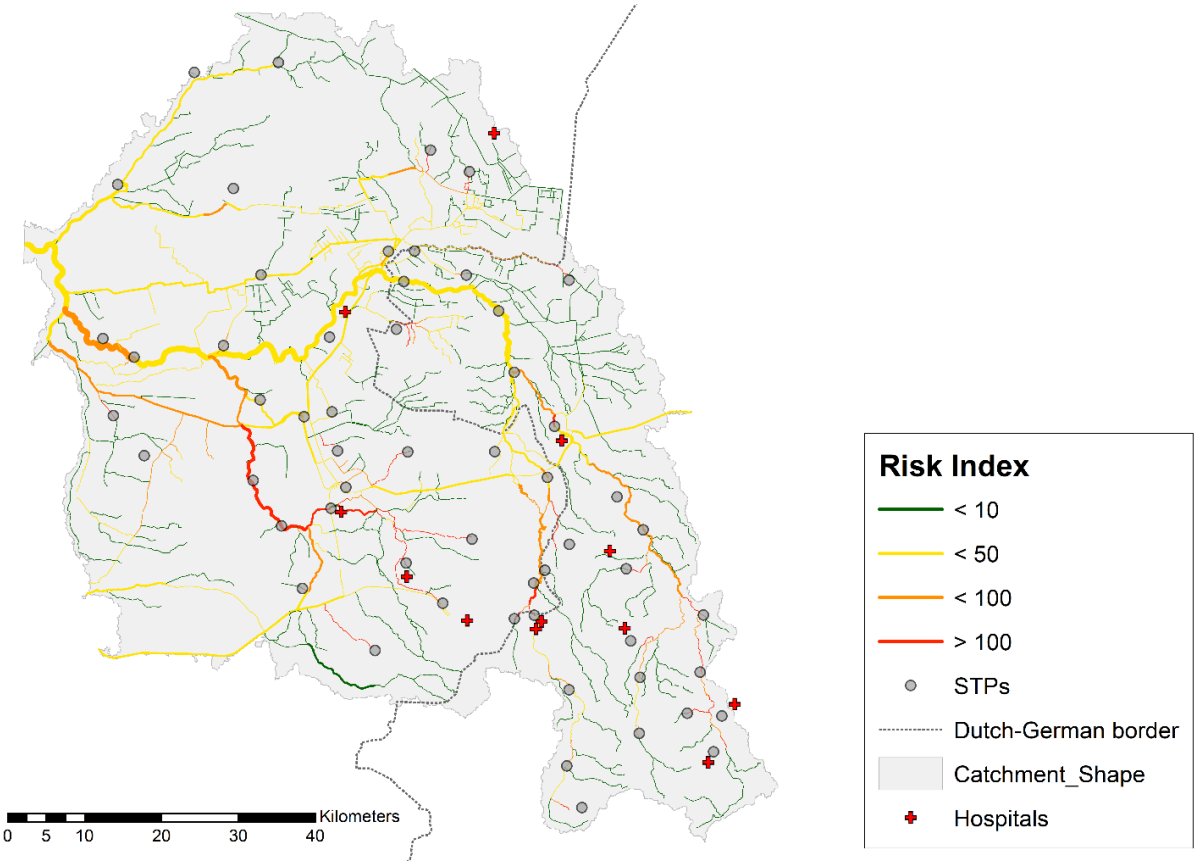

Figure B.5: Risk index map of the Vecht River catchment during a typical dry-summer-scenario. Dashed line demarks the German-Dutch border.

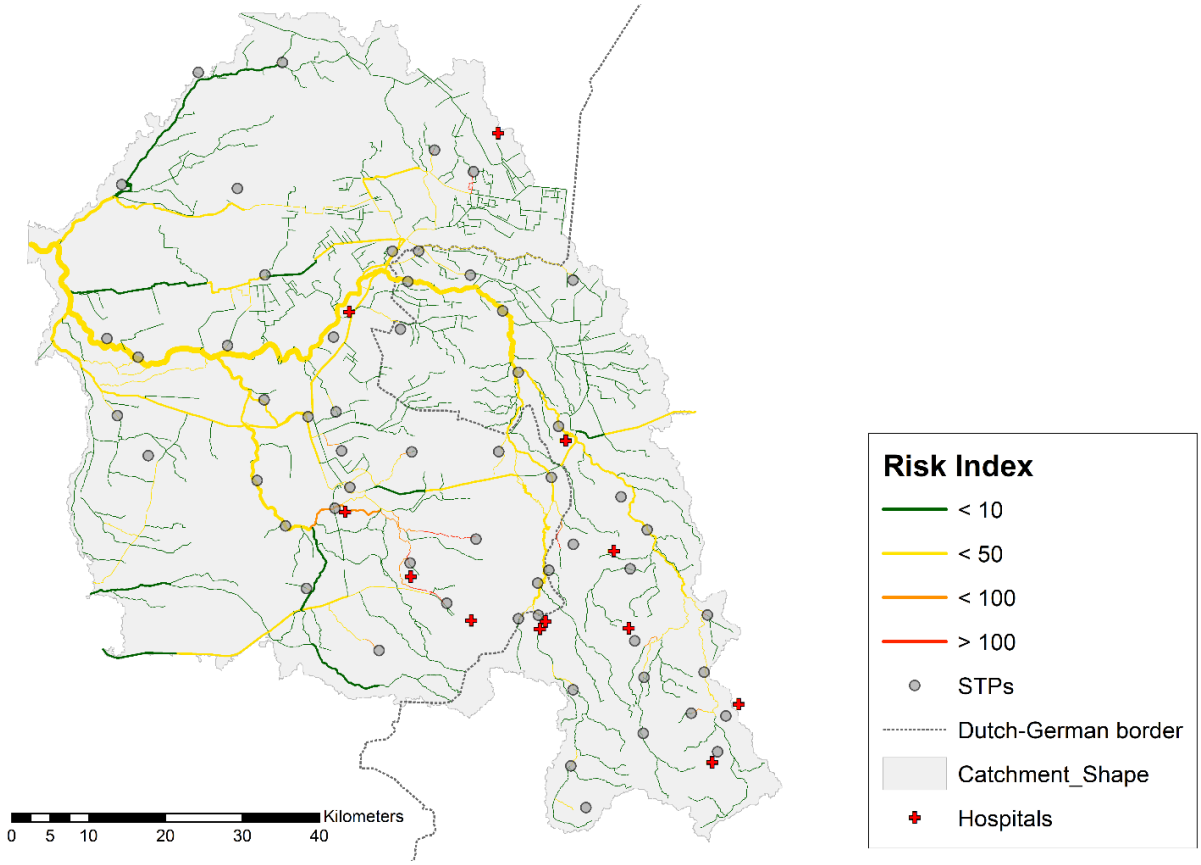

Figure B.6: Risk index map of the Vecht River catchment during a typical average-condition-scenario. Dashed line demarks the German-Dutch border.

C. Appendix to Chapter 6

List of supporting texts

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Table C.1: Land use in the Vecht catchment. Data is summarized from CORINE Land Cover (European Environment Agency, 2018).

Land use	Share	Comment
Arable land	27.1%	Non-irrigated arable land
Forest	9.7%	
Grasland, shrubs, transitional land	1.5%	
Mixed land use	17.7%	Principally consists of cultivated areas and pastures with areas of natural vegetation and scattered houses or gardens
Pastures	33.2%	Pastures, meadows and other permanent grasslands under agricultural use
Surface waters	0.3%	
Urban	9.5%	Cities, roads, industry, roads, rail networks, airports, mineral extraction sites and dump sites
Wetland	1.0%	

Text 1: Sampling procedure

WWTP operators provided 24-h samples of WWTP influent and effluent. Some of the plants provided flow proportional samples. Surface water samples were taken according to NEN 6600-2 (NEN, 2009) using a sampling stick with 1 L beaker or a 10 L bucket dropped down from a bridge. Sampling time, weather, water temperature and circumstances were noted. Samples were cooled during transportation, stored at 4 °C and processed within 24 hours.

Text 2: Quantification methods

For isolation of *E. coli*, ESBL-producing *E. coli* (ESBL-EC) and carbapenemase-producing *E. coli* (CP-EC), water samples were filtered through a membrane filter with a pore size of 0.45 µm (Merck, Amsterdam, the Netherlands) according to ISO 8199:2018 (ISO, 2018). Different dilutions and volumes (ranging from 10 mL to 300 mL) were used depending on the expected bacterial concentration of the different sample types.

After filtration, the filters were placed on selective agar plates and incubated for 4 hours at 37 °C and 18–24 hours at 44 °C. To quantify *E. coli*, Tryptone Bile X-glucuronide agar (TBX) (EWC Diagnostics, Steenwijk, The Netherlands; ref. T703.02) was used in accordance with ISO 16649–2 (ISO, 2001). For quantification of resistant bacteria, the following agar plates were used: ChromID ESBL (BioMérieux, Amersfoort, the Netherlands; ref. 43481) to detect ESBL-EC and ChromID CARBA (BioMérieux; ref. 43861) to detect CP-EC (i.e. focus was laid on expected CP-EC genotypes other than OXA-48). All samples were analyzed for *E. coli* and ESBL-EC, while CP-EC were only cultured from influent and effluent samples. From the counts, concentrations in CFU L^{-1} were calculated according to ISO 8199 (ISO, 2018), except that counts with a total number of less than 10 colonies were also included for ESBL-EC and CP-EC. From every ESBL and CARBA plate, 5–10 colonies were confirmed with an indole test for species identity. A selection of the colonies was also subjected to species identification and confirmation of phenotypic ESBL resistance by VITEK (BioMérieux, Amersfoort, the Netherlands) and combination disk test (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf).

92 out of 92 tested isolates (100%) from ChromID ESBL plates showed a phenotype indicative of extended spectrum beta lactamase (ESBL) production. A selection of CP-EC (192) was also subjected to WGS for confirmation of species and CP gene carriage.

Table C.2: Sampling site coordinates of the monitoring campaign.

Sample type	Sampling site ID	Coordinates (Latitude, Longitude)	Relative to WWTP (only longitudinal profile)
WWTP	W01	N52°32'27.48", E6°36'23.41"	
WWTP	W02	N52°14'02.8", E6°50'36.8"	
WWTP	W03	N52°9'46.95", E7°20'2.09"	
WWTP	W04	N52°26'43.89", E7°2'43.88"	
WWTP	W05	N52°24'43.9", E6°55'46.7"	
WWTP	W06	N52°13'27.2", E7°00'49.9"	
WWTP	W07	N52°19'35.9", E7°13'13.0"	
WWTP	W09	N52°20'28.9", E6°37'31.6"	
WWTP	W10	N52°30'37.3", E6°14'14.5"	
WWTP	W11	N52°26'48.6", E6°33'56.0"	
Longitudinal profile	H00	N52°33'22.55", E6°36'33.17"	1.6 km upstream
Longitudinal profile	H01	N52°32'36.22", E6°35'13.93"	0.5 km downstream
Longitudinal profile	H02	N52°32'21.23", E6°35'8.39"	1.0 km downstream
Longitudinal profile	H03	N52°31'23.88", E6°34'4.31"	3.5 km downstream
Longitudinal profile	H04	N52°30'55.14", E6°32'51.51"	5.4 km downstream
Longitudinal profile	H05	52°30'45.61", E6°30'53.80"	8.8 km downstream
Longitudinal profile	H06	N52°31'40.48", E6°29'54.87"	11.0 km downstream
Longitudinal profile	H07	N52°31'1.43", E6°25'25.47"	16.8 km downstream
Longitudinal profile	S00	N52°9'21.51", E7°20'10.01"	1.0 km upstream
Longitudinal profile	S01	N52°10'39.61", E7°20'13.40"	2.4 km downstream
Longitudinal profile	S02	N52°10'57.56", E7°20'0.50"	3.4 km downstream
Longitudinal profile	S03	N52°11'36.30", E7°19'46.01"	5.0 km downstream
Longitudinal profile	S04	N52°12'20.67", E7°19'26.93"	6.6 km downstream
Longitudinal profile	S05	N52°12'54.03", E7°19'27.01"	7.8 km downstream
Longitudinal profile	S06	N52°13'16.75", E7°19'49.16"	9.0 km downstream

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Table C.2 – continued from previous page

Sample type	Sampling site ID	Coordinates (Latitude, Longitude)	Relative to WWTP (only longitudinal profile)
Background site	B02	N52°3'55.81", E7°6'11.57"	
Background site	B04	N52°12'33.99", E6°58'35.98"	
Background site	B07	N52°23'25.72", E7°9'40.89"	
Background site	B10	N52°28'24.25", E6°44'15.97"	
Background site	B13	N52°39'22.76", E6°39'16.59"	
Background site	B17	N52°14'35.84", E6°40'36.44"	
Background site	B18	N52°24'43.51", E6°28'26.56"	
General catchment site	G01	N52°2'46.76", E7°4'49.56"	
General catchment site	G02	N52°7'1.37", E7°20'9.30"	
General catchment site	G03	N52°13'16.75", E7°19'49.16"	
General catchment site	G04	N52°16'52.76", E7°13'10.75	
General catchment site	G05	N52°14'7.75", E7°0'12.35"	
General catchment site	G07	N52°19'6.48", E6°45'42.91"	
General catchment site	G08	N52°18'25.62", E6°35'8.79"	
General catchment site	G09	N52°44'35.29", E6°47'22.29"	
General catchment site	G10	N52°13'27.40", E6°34'48.60"	
General catchment site	G11	N52°36'38.35", E6°43'31.05"	

Table C.3: Characteristics of the ten monitored wastewater treatment plants (WWTPs) in the Vecht catchment.

Sampling site ID	Country	Connected population	Receiving hospital wastewater	Advanced treatment techniques	Nr of sampling events
W01 [a]	NL	32 050	Yes		10
W02	NL	179 917	Yes		10
W03 [a]	GE	14 712			9
W04	GE	128 300	Yes		10
W05	NL	9 233		Hybrid MBR [b]	10
W06	GE	47 269	Yes		9
W07	GE	30 600			10
W09	NL	95 167	Yes		10
W10	NL	26 390			10
W11	NL	18 550		Hybrid Nereda [c]	10

[a] WWTPs that were selected for downstream concentration profile measurement.
[b] Up to 50% of receiving wastewater is treated by a membrane bio-reactor.
[c] A parallel operating activated sludge system is fed with Nereda® waste sludge which settles more easily

Table C.4: Land use upstream of background sites. Land use classes refer to Table C.1.

Monitoring site	Arable land	Pastures	Mixed land use	Forest	Other land use classes
B02	85.3%	5.9%	0.0%	6.3%	2.5%
B04	33.4%	37.1%	3.5%	7.7%	18.3%
B07	77.8%	6.5%	0.0%	15.6%	0.1%
B10	58.2%	10.6%	0.0%	25.3%	5.9%
B13	68.5%	12.0%	5.4%	0.0%	14.1%
B17	7.2%	21.4%	50.9%	18.8%	1.7%
B18	5.4%	55.3%	25.2%	2.2%	11.9%

Table C.5: Sampling dates of all WWTP samples. On every sampling date, both an influent and an effluent sample were taken. Eight WWTPs were sampled from July 2018 on and sampling of another two WWTPs started in December 2018. In January 2019, due to practical circumstances, no samples were taken except for the German WWTPs. In the months February to May 2019, some WWTPs were not able to provide samples for a variety of reasons. These WWTPs provided an extra sample in the month June.

Sampling date	W09	W10	W02	W06	W01	W04	W05	W07	W03	W11
2018-07-18	2	0	0	0	2	2	2	2	0	2
2018-07-23	0	2	0	0	0	0	0	0	0	0
2018-07-25	0	0	2	0	0	0	0	0	0	0
2018-08-07	0	2	2	0	0	0	2	0	0	0
2018-08-14	0	0	0	0	2	0	0	0	0	2
2018-08-22	0	0	0	0	0	2	0	2	0	0
2018-08-30	2	0	0	0	0	0	0	0	0	0
2018-09-10	0	0	0	0	0	0	0	2	0	0
2018-09-11	0	2	0	0	2	2	0	0	0	2
2018-09-18	2	0	2	0	0	0	2	0	0	0
2018-10-09	0	2	0	0	2	0	0	0	0	2
2018-10-17	0	0	0	0	0	2	0	2	0	0
2018-10-18	2	0	2	0	0	0	2	0	0	0
2018-11-06	2	0	2	0	0	0	2	0	0	0
2018-11-13	0	2	0	0	2	0	0	0	0	2
2018-11-14	0	0	0	0	0	2	0	2	0	0
2018-12-04	0	2	0	0	2	0	0	0	0	2
2018-12-05	0	0	0	2	0	2	0	2	0	0
2018-12-10	0	0	0	0	0	0	0	0	2	0
2018-12-13	2	0	2	0	0	0	2	0	0	0
2019-01-09	0	0	0	2	0	2	0	2	2	0

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Table C.5 – continued from previous page

Sampling date	W09	W10	W02	W06	W01	W04	W05	W07	W03	W11
2019-02-07	2	0	2	0	0	0	2	0	0	0
2019-02-13	0	2	0	0	2	0	0	0	0	0
2019-02-18	0	0	0	0	0	0	0	0	2	0
2019-02-20	0	0	0	2	0	2	0	2	0	0
2019-03-05	0	2	0	0	2	0	0	0	0	2
2019-03-06	0	0	0	0	0	0	0	0	2	0
2019-03-11	0	0	0	2	0	2	0	2	0	0
2019-03-14	2	0	0	0	0	0	2	0	0	0
2019-04-04	0	2	0	0	2	0	0	0	0	2
2019-04-09	0	0	2	0	0	0	2	0	0	0
2019-04-10	0	0	0	0	0	0	0	0	2	0
2019-04-15	0	0	0	2	0	2	0	2	0	0
2019-05-06	0	0	0	0	0	0	0	0	2	0
2019-05-14	2	0	2	0	0	0	2	0	0	0
2019-05-15	0	0	0	2	0	0	0	0	0	0
2019-05-21	0	0	0	0	2	0	0	0	0	2
2019-06-25	2	2	2	2	0	0	0	0	2	2
2019-07-22	0	0	0	2	0	0	0	0	2	0
2019-08-19	0	0	0	2	0	0	0	0	2	0

Table C.6: Sampling dates of surface water samples (background, general catchment samples and longitudinal profiles).

Sampling date	B02	B04	B07	B10	B13	B17	B18	G01	G02	G04	G05	G07	G08	G09	G10	G11	W01 Profile	W03 Profile
2018-07-16	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2018-07-18	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2018-07-23	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2018-07-25	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	6
2018-08-06	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2018-08-13	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2018-08-15	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2018-08-22	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	6
2018-09-10	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2018-09-12	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2018-09-19	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	7
2018-09-26	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2018-10-08	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2018-10-10	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2018-10-15	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	7
2018-10-22	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2018-11-05	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2018-11-07	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	7	0
2018-11-12	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	7
2018-11-14	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2018-12-03	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2018-12-05	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2018-12-10	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	6
2018-12-12	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0

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Table C.6 – continued from previous page

Sampling date	B02	B04	B07	B10	B13	B17	B18	G01	G02	G04	G05	G07	G08	G09	G10	G11	W01 Profile	W03 Profile
2019-02-11	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2019-02-13	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2019-02-18	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	6
2019-02-20	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2019-03-06	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	6
2019-03-11	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2019-03-13	1	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2019-03-18	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2019-04-01	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8	0
2019-04-03	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0	0
2019-04-10	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	6
2019-04-15	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
2019-05-06	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	5

Table C.7: Wastewater treatment plant (WWTP) data providers.

WWTP	Data providers
W01	Waterschap Vechtstromen
W02	Waterschap Vechtstromen
W03	LANUV [a], Kreisstadt Steinfurt
W04	NLWKN [b], Kommunale Betriebe Nordhorn
W05	Waterschap Vechtstromen
W06	LANUV [a], Stadtwerke Gronau
W07	NLWKN [b], Kommunale Betriebe Nordhorn
W09	Waterschap Vechtstromen
W10	Waterschap Drents Overijsselse Delta
W11	Waterschap Vechtstromen

[a] Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency.
[b] State Agency for Nature, Environment and Consumer Protection.

Table C.8: Response variables and explanatory variables used in the linear mixed models.

Response variables [a]	Explanatory variables						
	Country (Netherlands, Germany)	Seasons (summer, remaining year) [b]	Normalized WWTP discharge [c]	Bacteria (*E. coli*, ESBL-EC) [d]	Hospital wastewater (yes, no)	Matrix (influent, effluent)	WWTP (random factor)
$pcL_{E.coli,In}$	X	X	X				X
$logRed$	X	X	X	X			X
$r_{ESBL-EC}$	X	X			X	X	X
r_{CP-EC}	X	X			[e]	[d]	X

[a] Response variables: $pcL_{E.coli,In}$: per capita influent load of *E. coli*, $logRed$: reduction of bacteria during wastewater treatment, $r_{ESBL-EC}$: relative abundance of ESBL *E. coli* in wastewater, r_{CP-EC}: relative abundance of CP *E. coli* in wastewater.

[b] Summer in the Northern Hemisphere: June 21–September 22.

[c] Normalized by dry weather flow.

[d] Detection frequency of CP-EC in WWTP effluents was too low to calculate CP-EC reduction or to account for possible matrix effects.

[e] Detection frequency of CP-EC was too low in WWTPs not treating hospital effluents.

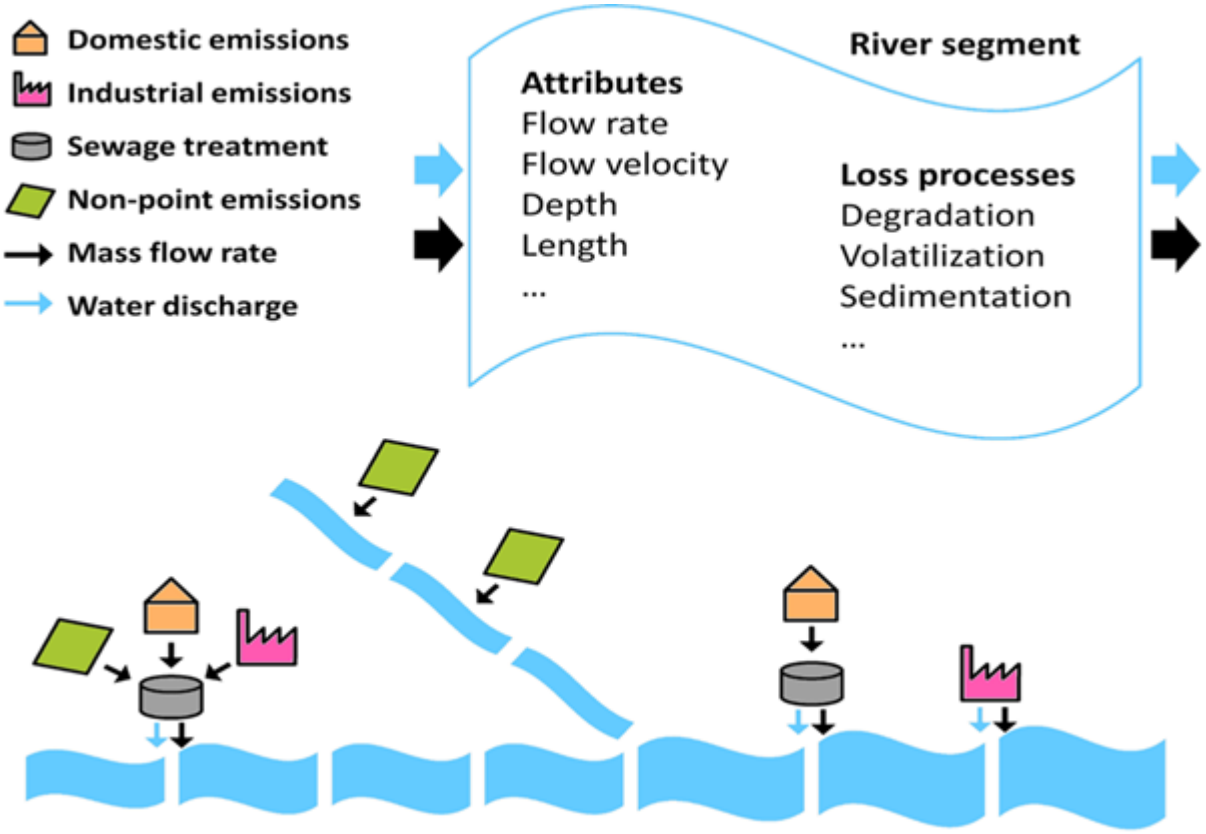

Figure C.1: Conceptual representation of the GREAT-ER model. Concentrations are calculated by dividing mass flow rates by the water discharge of a river segment.

Table C.9: Characteristics of the average flow scenario and the dry summer scenario (adopted from Duarte et al. (2021)).

	Average flow scenario	Dry summer scenario
Applicability	Humid periods throughout the whole year	Dry periods without rainfall between June and September
Flow rate at the border [m^3 s^{-1}]	18.5	2.8
Flow rate at the Zwarte Water [m^3 s^{-1}]	63.5	11.3
Pumping activity	No	Yes
Average water temperature [°C]	11.9	18.2

Table C.10: Fractions of *E. coli* attached to suspended materials in natural waterbodies. Median attached fraction: 36.5%.

Attached fraction [%]	Source	Matrix
34.0	Jamieson et al. (2005a)	Suspended sediment
20.0	Jamieson et al. (2005a)	Suspended sediment
44.0	Jamieson et al. (2005a)	Suspended sediment
27.0	Jamieson et al. (2005a)	Suspended sediment
30.0	Garcia-Armisen and Servais (2009)	Suspended matter
36.5	Characklis et al. (2005)	Suspended solids
37.5	Characklis et al. (2005)	Suspended solids
53.6	Characklis et al. (2005)	Suspended solids
38.0	Fries et al. (2006)	Suspended particles

Text 3: WWTP model Parameterization

Different parameter sets were used for the average flow scenario and the dry summer scenario depending on boundary conditions, e.g. season or country. Parameters were derived from statistical analysis with linear mixed models (Section 6.2.3). Final reduced models are presented in Table C.11 and parameterization of the models in Table C.12. Resulting input parameters for the GREAT-ER model are presented in Table C.13. For the model evaluation (Section 6.3.4), all WWTPs are parametrized by the same per capita influent load and WWTP reduction; i.e. monitored WWTPs are parametrized in the same way as non-monitored WWTPs to evaluate the applicability of a generalized model. For the exposure assessment (Section 6.3.5) however, monitored WWTPs are parametrized based on on-site measurement data to include best available information.

To illustrate the WWTP model Parameterization an example is provided: Calculation of *E. coli* loads in WWTP effluents in WWTP W01 (32 050 inhabitants) for the dry summer scenario. Daily effluent loads (L_{eff} [CFU d^{-1}]) are calculated as

$$L_{eff} = pcL_{in} \times Inh \times 10^{-logRed} \tag{C.1}$$

Where pcL_{in} is the per capita influent load [CFU cap^{-1} d^{-1}] of *E. coli*, Inh [cap] is the number of inhabitants connected to the WWTP and $logRed$ is the logarithmic reduction of bacterial loads by wastewater treatment. pcL_{in} of *E. coli* is calculated with model 2 (Table C.11):

$$\log pcL_{in} = 10.206 + 0.352 \times X_1 \tag{C.2}$$

where X_1 is 1 for summer and 0 for the remaining year. This results in $\log pcL_{in} = 10.558$ and leads to $pcL_{in} = 3.61 \times 10^{10}$ CFU cap^{-1} d^{-1}. logRed is calculated with model 4 (Table C.11):

$$logRed = 3.142 + 0.305 \times X_1 - 0.399 \times X_2 \tag{C.3}$$

where X_1 is 1 for summer and 0 for the remaining year and X_2 is the normalized WWTP discharge, i.e. discharge normalized by dry weather flow (DWF). This indicates that treatment efficiency is highest, when WWTP discharge is low and when it is summer. For the dry summer scenario we assume that the discharge is equal to the DWF for all WWTPs in summer. This leads to

$$logRed = 3.142 + 0.305 \times 1 - 0.399 \times 1 = 3.048 \tag{C.4}$$

The fully parametrized WWTP emission model for *E. coli* in the dry summer scenario for WWTP W01 is then:

$$L_{eff} = 3.61 \times 10^{10} \times 32\ 050 \times 10^{-3.048} = 1.04 \times 10^{12} \tag{C.5}$$

In the dry summer scenario, WWTP W01 is predicted to release 1.04×10^{12} CFU of *E. coli* per day into the receiving river.

Table C.11: Linear mixed models. Full and reduced models. Reduced models only consist of variables that were found to be significant in the full models. Acronyms and abbreviations: log.load: *E. coli* influent load per inhabitant. Season: divided into summer and remaining year. Country: Germany and the Netherlands. WWTP: Monitored wastewater treatment plant. log.red: log reduction of bacteria in WWTP. Bacteria: *E. coli* and ESBL *E. coli* (ESBL-EC); detection frequency of carbapenemase-producing *E. coli* (CP-EC) was too low in effluent to calculate removal efficiencies. log.ratio.esbl: log of ESBL-EC to *E. coli* ratio. log.ratio.cpec: log of CP-EC to *E. coli* ratio. Hospital: WWTP treats hospital wastewater. Matrix: Influent and effluent.

Variable	Model number	Model type	Model formula
E. coli influent	1	Full	log.load ~ Season + Country + Q.norm + (1—WWTP)
	2	Reduced	log.load ~ Season + (1—WWTP)
Removal	3	Full	log.red ~ Season + Country + Q.norm + Bacteria + (1—WWTP)
	4	Reduced	log.red ~ Season + Q.norm + (1—WWTP)
ARB ratio	5	Full	log.ratio.esbl ~ Season + Country + Hospital + Matrix + (1—WWTP)
	6	Reduced	log.ratio.esbl ~ Season + Country + (1—WWTP)
	7	Full	Log.ratio.cpec ~ Season + Country + (1—WWTP)
	8	Reduced	Log.ratio.cpec ~ (1—WWTP)

Table C.12: Parameterization of reduced linear mixed models. Acronyms and abbreviations: log.load: log of *E. coli* per capita load in WWTP influent. Season: divided into summer and remaining year. Country: Germany (GE) and the Netherlands (NL). WWTP: Monitored wastewater treatment plant. log.red: log reduction of bacteria in WWTP. log.ratio.esbl: log of ESBL-EC to *E. coli* ratio. log.ratio.cpec: log of CP-EC to *E. coli* ratio.

Model number	Model formula	Factor	Intercept/Beta	SE	p	CI lower	CI upper
2	log.load ∼ Season + (1—WWTP)	Intercept	10.206	0.036	< 0.001	10.129	10.283
		Season summer	0.352	0.048	< 0.001	0.258	0.446
4	log.red ∼ Season + Q.norm + (1—WWTP)	Intercept	3.142	0.188	< 0.001	2.745	3.541
		Season summer	0.305	0.097	< 0.01	0.115	0.495
		Q.norm	−0.399	0.047	< 0.001	−0.491	−0.307
6	log.ratio.esbl ∼ Season + Country + (1—WWTP)	Intercept	−1.796	0.055	< 0.001	−1.914	−1.678
		Season summer	0.139	0.042	< 0.001	0.057	0.221
		Country NL	−0.324	0.069	< 0.001	−0.475	−0.174
8	Log.ratio.cpec ∼ (1—WWTP)	Intercept	−5.291	0.190	< 0.001	−5.813	−4.905

Table C.13: GREAT-ER model input parameters. pcL_{in}: Per capita influent load, *logRed*: log reduction of bacteria in WWTP. GE: Germany, NL: Netherlands.

Parameter	Bacteria	Scenario	Country	Value	Comment
pcL_{in}	*E. coli*	Average flow scnenario	GE, NL	1.61×10^{10} [CFU cap^{-1} d^{-1}]	Calculated with model 2
pcL_{in}	ESBL *E. coli*	Average flow scnenario	GE	2.57×10^{8} [CFU cap^{-1} d^{-1}]	ESBL *E. coli* to *E. coli* ratio calculated with model 6 is applied to per capita load of *E. coli* calculated with model 2
pcL_{in}	ESBL *E. coli*	Average flow scnenario	NL	1.22×10^{8} [CFU cap^{-1} d^{-1}]	ESBL *E. coli* to *E. coli* ratio calculated with model 6 is applied to per capita load of *E. coli* calculated with model 2
pcL_{in}	CP *E. coli*	Average flow scnenario	GE, NL	8.22×10^{4} [CFU cap^{-1} d^{-1}]	CP *E. coli* to *E. coli* ratio calculated with model 8 is applied to per capita load of *E. coli* calculated with model 2
pcL_{in}	*E. coli*	Dry summer scenario	GE, NL	3.61×10^{10} [CFU cap^{-1} d^{-1}]	Calculated with model 2
pcL_{in}	ESBL *E. coli*	Dry summer scenario	GE	7.96×10^{8} [CFU cap^{-1} d^{-1}]	ESBL *E. coli* to *E. coli* ratio calculated with model 6 is applied to per capita load of *E. coli* calculated with model 2
pcL_{in}	ESBL *E. coli*	Dry summer scenario	NL	3.78×10^{8} [CFU cap^{-1} d^{-1}]	ESBL *E. coli* to *E. coli* ratio calculated with model 6 is applied to per capita load of *E. coli* calculated with model 2
pcL_{in}	CP *E. coli*	Dry summer scenario	GE, NL	1.85×10^{5} [CFU cap^{-1} d^{-1}]	CP *E. coli* to *E. coli* ratio calculated with model 8 is applied to per capita load of *E. coli* calculated with model 2
logRed	*E. coli*, ESBL *E. coli*, CP *E. coli*	Average flow scnenario	GE, NL	Each WWTP individually, depending on Q.norm: *logRed* = 3.142 - 0.399 x Q.norm [-]	Calculated with model 4; Q.norm is calculated as the ratio of average daily discharge to dry weather flow of the respective WWTP
logRed	*E. coli*, ESBL *E. coli*, CP *E. coli*	Dry summer scenario	GE, NL	3.048 [-]	Calculated with model 4; Q.norm is equal to 1

Text 4: Background sites - monitoring and model Parameterization

E. coli concentrations in background samples ranged over almost four orders of magnitude (0.81–4.61 log CFU L^{-1} (median 3.17 log CFU L^{-1}). Due to lower concentrations - often close to or below the detection limit, the range of ESBL concentrations was smaller (< LOQ – 2.18 log CFU L^{-1}). The median concentration of positive samples was 0.70 log CFU L^{-1}. Relative abundance of ESBL-EC was lower in background sites (0.14%) as compared to WWTP effluents by approximately one order of magnitude. Three background locations (i.e. B02, B04, B07) have a relatively high *E. coli* concentration and detection rate of ESBL-EC compared to the other background sites, for which we could find no obvious reasons. Blaak et al. (2018) measured median *E. coli* and ESBL-EC concentrations of 1.5×10^3 and 5.7 CFU L^{-1}, respectively, with 32% of ESBL-EC above the LOQ during a 9-month sampling campaign at an agricultural monitoring site without WWTP influence.

In the model, bacterial concentrations C [CFU L^{-1}] in river flow increments ΔQ are defined to estimate the respective diffuse emission loads (Section 6.2.4). In a calibration step, these concentrations were adjusted so that measured concentrations at background sampling sites best agreed with the simulation results. This results in increment concentrations of 4.5×10^3 and 3.1×10^4 CFU L^{-1} in the average flow scenario and the dry summer scenario, respectively (Table C.14).

Due to the large number of non-detects (see Figure C.2), Parameterization of the ESBL-EC increment concentrations was based on *E. coli* using relative abundance of ESBL-EC. We assume that ESBL-EC to *E. coli* ratios are always the same in all river flow increments. From measured data at the background monitoring sites a median value of 0.14% was derived for this ratio. For CP-EC no such data was available. Therefore, diffuse emissions of CP-EC were not considered.

For this study, diffuse emissions of bacteria are thought to encompass (i) passive transport by the flow components runoff, interflow, baseflow and (ii) remobilization of bacteria from the sediments. These processes are thought to contribute differently to diffuse emissions and background concentrations in the two modeled scenarios. The exact quantification of the contribution of individual processes however, cannot be provided here due to insufficient data and process understanding.

In the dry summer scenario, where mainly groundwater exchange is responsible for river flow, diffuse emissions of bacteria are thought to mainly account for remobilization of bacteria from the sediments. Sediments are a reservoir for *E. coli* bacteria (Pachepsky and

Shelton, 2011). The work by Kim et al. (2010) indicates that sediment concentrations of *E. coli* bacteria are 2 to 3 orders of magnitude higher in summer and autumn compared to the remaining year. The bacteria in the sediment reservoir can be mobilized by groundwater flowing into the river (Pachepsky et al., 2017) but also by active mobilization (Park et al., 2017). Pachepsky et al. (2017) even observed an increase in *E. coli* concentrations under base flow conditions.

In the average flow scenario, the remobilization of bacteria from the sediments is thought to additionally appear due to bed shear stress due to high flows (Jamieson et al., 2005b). Considering the work of Kim et al. (2010), (2–3 orders of magnitude higher *E. coli* concentrations in the sediments in summer and autumn), the total load entering the water column by remobilization is thought to be lower compared to the dry summer scenario. In contrast to the dry summer scenario, the flow in the average flow scenario also consists of interflow and runoff. Especially the latter contributes to diffuse emissions of *E. coli* in the Vecht catchment, which is characterized by agricultural activities. Due to the manure application on arable land and grassland as well as livestock on pasture land, fecal bacteria, i.e. *E. coli*, are introduced to agricultural areas. Here, they can survive several months before they are transported by surface flow or washed off into adjacent rivers (Avery et al., 2004).

Table C.14: Parameterization of bacterial concentrations C [CFU L^{-1}] in river flow increments ΔQ.

Scenario	*E. coli*	ESBL *E. coli*	CP *E. coli*
Dry summer	3.1×10^4	43.4	n.a. [a]
Average flow	4.5×10^3	6.3	n.a. [a]

[a] Not applied: Diffuse emissions are neglected for CP *E. coli* due to insufficient data.

Table C.15: Descriptive statistics for *E. coli* concentrations in WWTP influents and effluents. DF: Detection frequency. n = 10 for all WWTPs, except for W03 and W06 (n = 9).

Sampling site ID	Influent (log CFU L^{-1})					Effluent (log CFU L^{-1})				
	Mean	Median	Min	Max	DF	Mean	Median	Min	Max	DF
W01	8.03	7.84	7.31	8.38	100%	6.15	6.12	5.64	6.52	100%
W02	8.14	8.09	7.55	8.48	100%	4.84	4.86	4.13	5.15	100%
W03	7.73	7.61	6.96	8.19	100%	5.25	4.85	4.06	5.86	100%
W04	8.15	8.03	7.55	8.48	100%	5.09	4.60	4.20	5.68	100%
W05	7.97	7.99	7.38	8.32	100%	5.58	4.52	3.83	6.36	100%
W06	7.90	7.76	7.26	8.26	100%	6.01	5.83	3.66	6.37	100%
W07	8.17	8.14	7.70	8.47	100%	4.82	4.69	3.96	5.38	100%
W09	8.02	8.03	6.69	8.30	100%	5.93	5.23	4.50	6.83	100%
W10	7.96	7.80	7.55	8.24	100%	5.45	5.21	3.66	6.00	100%
W11	8.06	8.00	7.61	8.34	100%	6.15	5.81	5.30	6.77	100%

Table C.16: Descriptive statistics for ESBL *E. coli* concentrations in WWTP influents and effluents. DF: Detection frequency. n = 10 for all WWTPs, except for W03 and W06 (n = 9).

Sampling site ID	Influent (log CFU L^{-1})					Effluent (log CFU L^{-1})				
	Mean	Median	Min	Max	DF	Mean	Median	Min	Max	DF
W01	6.01	5.66	4.89	6.40	100%	3.95	3.84	3.20	4.36	100%
W02	6.36	6.30	5.54	6.79	100%	2.97	2.87	2.56	3.30	100%
W03	5.89	5.84	5.29	6.14	100%	3.49	3.09	2.40	4.05	100%
W04	6.47	6.37	5.78	6.86	100%	3.07	2.95	2.26	3.49	100%
W05	5.95	5.79	4.54	6.48	100%	3.24	2.42	1.36	3.99	100%
W06	6.05	5.86	5.47	6.46	100%	4.30	4.24	3.36	4.70	100%
W07	6.44	6.45	5.79	6.78	100%	3.05	2.97	2.53	3.40	100%
W09	6.17	6.17	4.80	6.47	100%	4.17	3.28	2.13	5.11	100%
W10	5.90	5.64	5.17	6.39	100%	3.34	3.09	1.66	3.99	90%
W11	6.07	5.75	5.44	6.55	100%	4.03	3.87	3.13	4.58	100%

Table C.17: Descriptive statistics for CP *E. coli* concentrations in WWTP influents and effluents. DF: Detection frequency. n = 10 for all WWTPs, except for W03 and W06 (n = 9).

Sampling site ID	Influent (log CFU L^{-1})					Effluent (log CFU L^{-1})				
	Mean	Median	Min	Max	DF	Mean	Median	Min	Max	DF
W01	2.98	2.98	1.62	3.27	20%	1.10	1.10	1.10	1.10	10%
W02	3.54	2.76	1.62	4.21	90%	[a]	[a]	[a]	[a]	0%
W03	2.62	2.62	2.62	2.62	11%	0.70	0.70	0.70	0.70	11%
W04	3.26	2.70	1.62	3.81	90%	0.94	0.94	0.40	1.18	20%
W05	[a]	[a]	[a]	[a]	0%	[a]	[a]	[a]	[a]	0%
W06	3.28	3.22	1.92	3.68	89%	1.73	1.35	1.23	2.25	56%
W07	1.51	1.51	1.36	1.62	20%	[a]	[a]	[a]	[a]	0%
W09	3.40	3.00	2.10	3.94	90%	2.14	1.18	0.40	2.81	50%
W10	2.46	2.46	2.46	2.46	10%	1.00	1.00	1.00	1.00	10%
W11	1.62	1.62	1.62	1.62	10%	0.40	0.40	0.40	0.40	10%

[a] < LOQ

Table C.18: Descriptive statistics for bacterial concentrations at background sites. DF: Detection frequency. n = 10 for all sampling sites.

Sampling site ID	*E. coli* (log CFU L^{-1})					ESBL *E. coli* (log CFU L -1)				
	Mean	Median	Min	Max	DF	Mean	Median	Min	Max	DF
B02	3.91	3.89	3.27	4.28	100%	1.33	0.40	0.39	1.98	60%
B04	4.03	3.77	2.82	4.61	100%	1.43	1.05	0.39	2.18	80%
B07	4.04	3.74	3.02	4.53	100%	1.04	0.57	0.40	1.52	60%
B10	3.22	2.29	0.81	4.11	100%	1.10	1.18	0.40	1.30	30%
B13	2.77	2.65	2.23	3.26	100%	0.40	0.40	0.40	0.40	10%
B17	3.38	3.05	2.17	3.84	100%	1.49	1.49	0.40	1.78	20%
B18	2.46	2.28	1.37	2.95	100%	0.88	0.88	0.88	0.88	10%

Table C.19: Descriptive statistics for bacterial concentrations at general catchment sites. DF: Detection frequency. n = 10 for all sampling sites.

Sampling site ID	*E. coli* (log CFU L^{-1})					ESBL *E. coli* (log CFU L^{-1})				
	Mean	Median	Min	Max	DF	Mean	Median	Min	Max	DF
G01	3.70	3.47	2.89	4.41	100%	2.20	1.30	1.18	2.99	70%
G02	4.35	4.03	3.91	4.95	100%	2.18	2.11	1.48	2.63	90%
G04	3.69	3.53	2.50	4.19	100%	2.22	2.09	1.72	2.64	50%
G05	4.32	4.06	3.72	4.68	100%	2.26	1.66	0.48	2.90	100%
G07	4.19	3.68	2.95	4.93	100%	2.12	1.87	0.70	2.81	80%
G08	3.34	3.26	2.82	3.71	100%	0.78	0.70	0.48	1.11	50%
G09	4.12	4.11	3.06	4.68	90%	2.17	2.00	1.11	2.64	100%
G10	3.05	2.09	1.36	3.75	100%	1.68	1.68	1.54	1.78	20%
G11	3.32	2.51	1.75	4.00	100%	1.78	1.66	0.48	2.16	60%

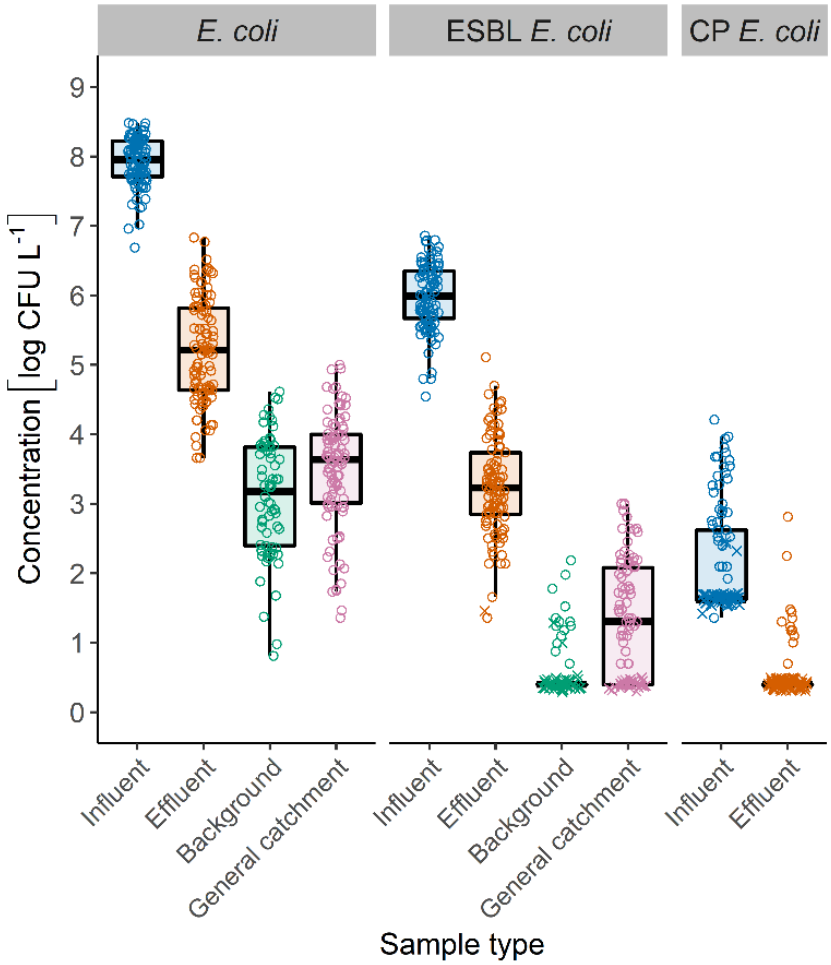

Figure C.2: Measured concentrations of *E. coli*, ESBL *E. coli* and CP *E. coli* bacteria in wastewater (influent and effluent samples) and surface water (background samples, general catchment samples). Crosses indicate concentrations below LOQ displayed as LOQ.

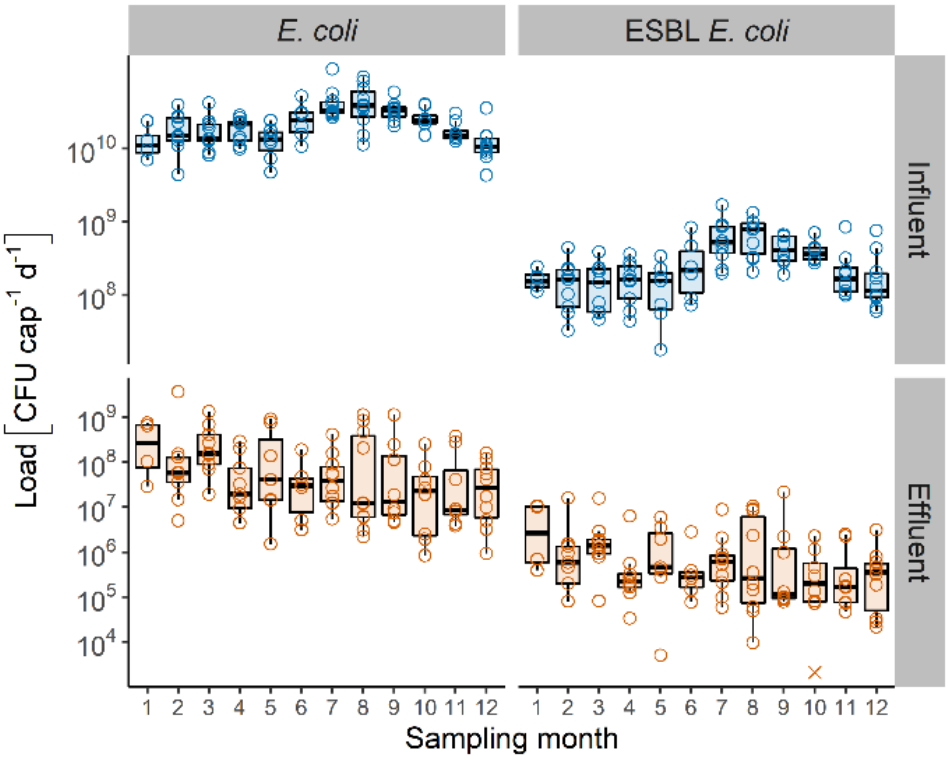

Figure C.3: Measured loads of *E. coli* and ESBL *E. coli* bacteria in wastewater (influent and effluent samples) over time. Crosses indicate concentrations below LOQ displayed as LOQ. January–June: 2019. July–December: 2018. July and August include two samples each from 2019. Influent loads exhibit a temporal trend with higher values in the period between June and October - i.e. mainly in summer - for *E. coli* as well as for ESBL-EC.

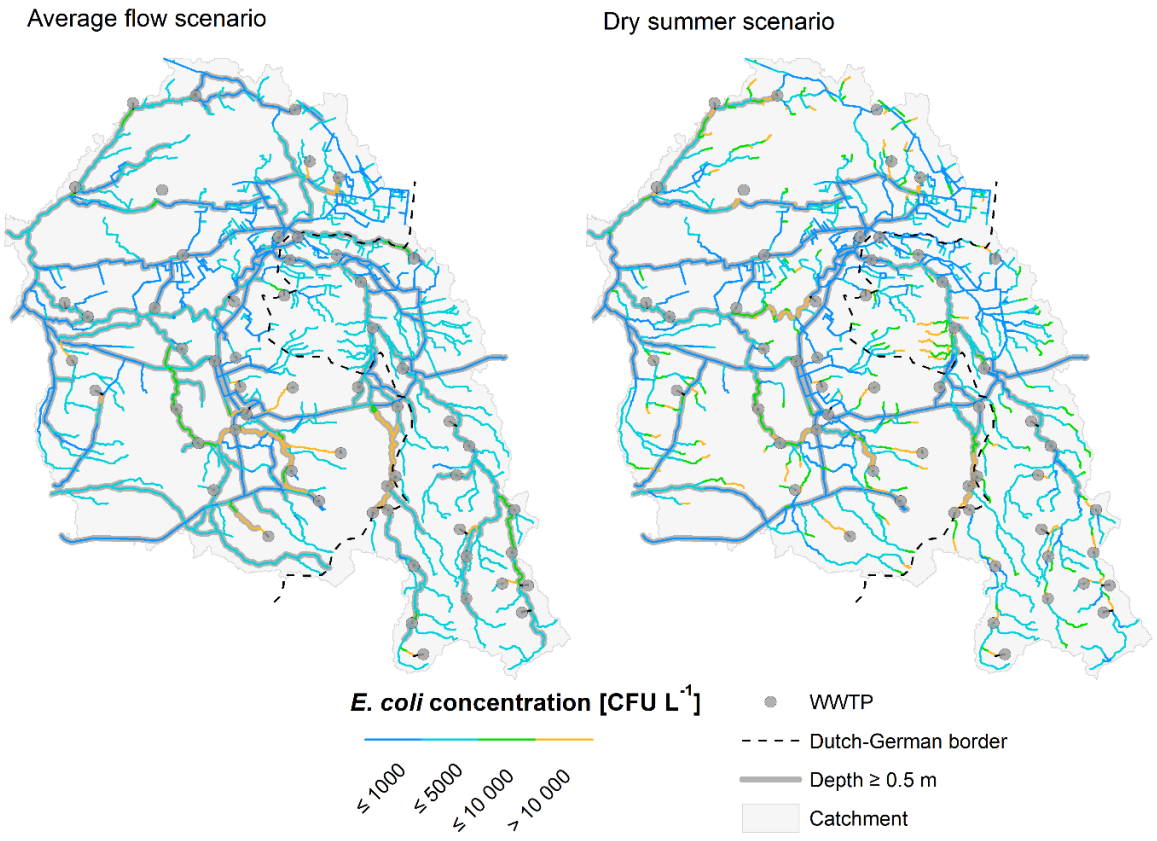

Figure C.4: Predicted concentration of *E. coli* in the average flow and the dry summer scenario. WWTP = wastewater treatment plant. A depth ≥ indicates potential swimming sites.

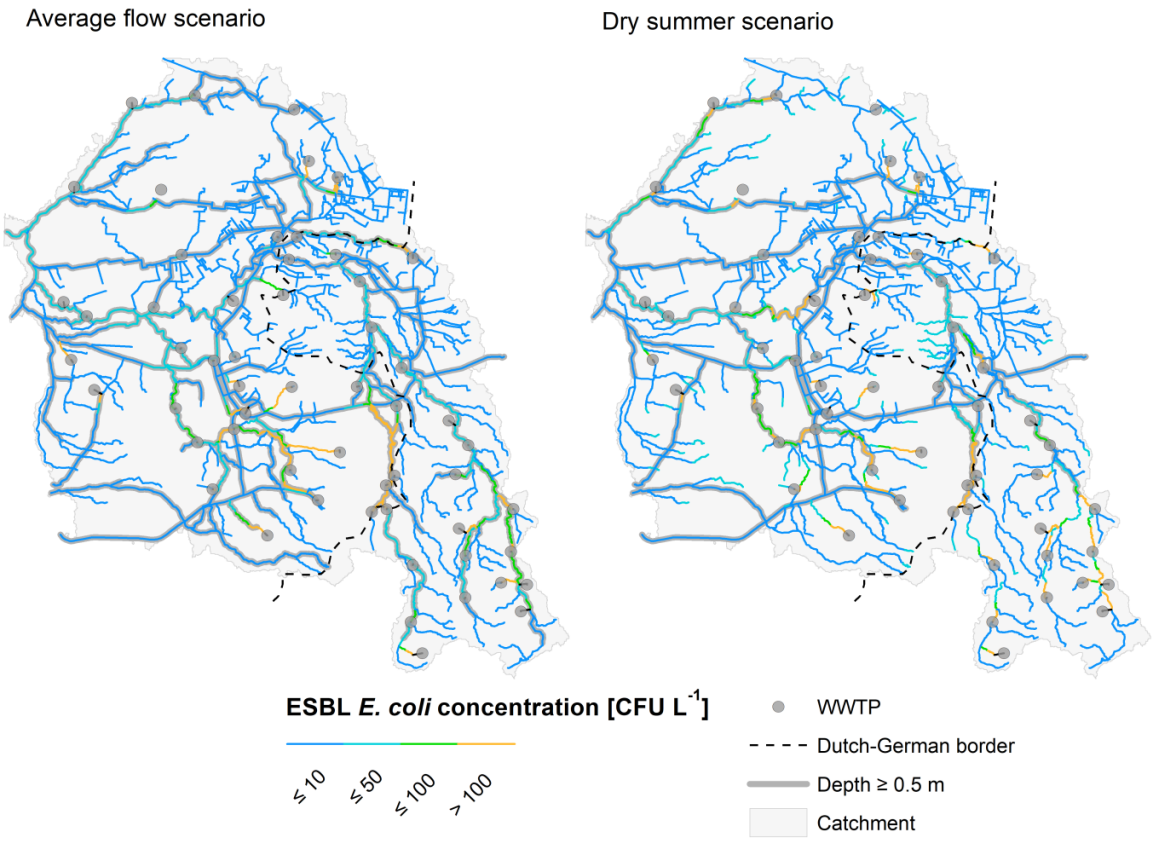

Figure C.5: Predicted concentration of ESBL *E. coli* in the average flow and the dry summer scenario. WWTP = wastewater treatment plant. A depth ≥ indicates potential swimming sites.

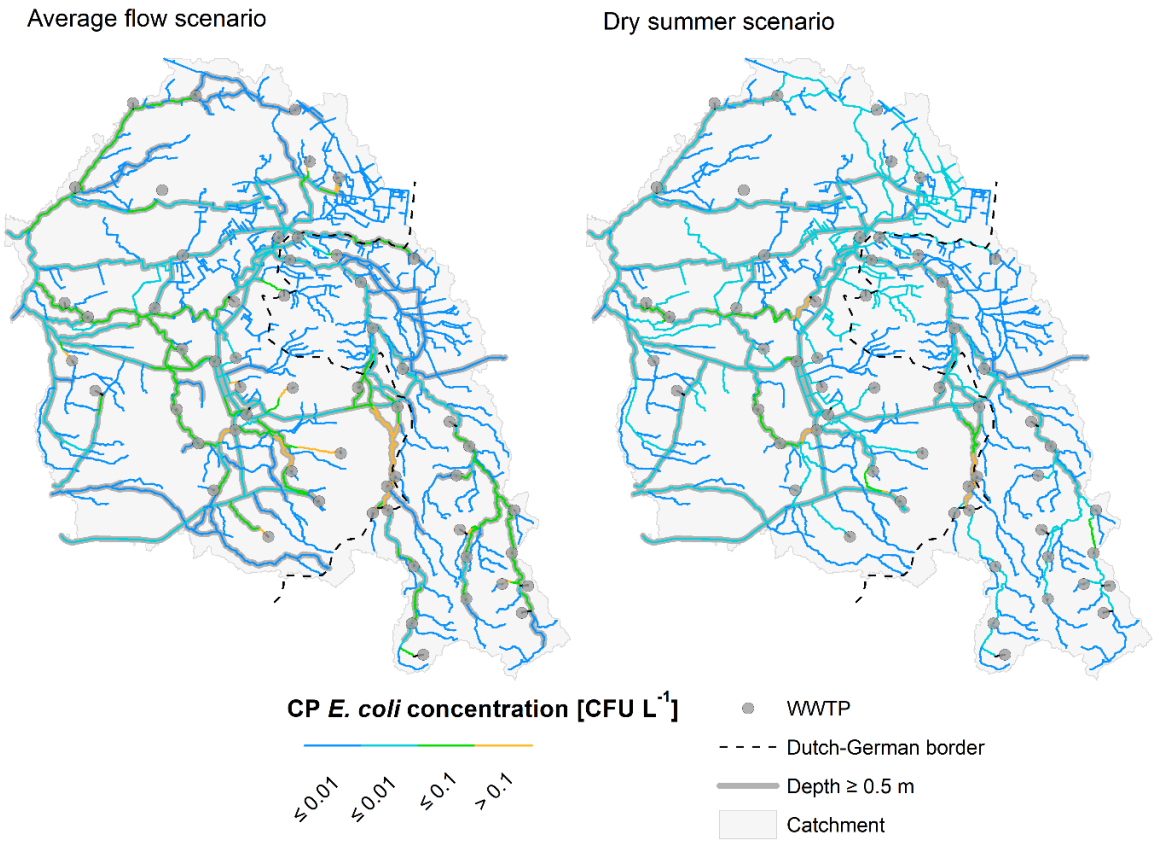

Figure C.6: Predicted concentration of CP *E. coli* in the average flow and the dry summer scenario. WWTP = wastewater treatment plant. A depth ≥ indicates potential swimming sites.

Text 5: Impact of modeled processes on *E. coli* concentrations

To assess the impact of WWTP emissions, diffuse emissions, sedimentation and inactivation on predicted *E. coli* concentrations we created scenarios where these processes were excluded. Thus, eight scenarios were created: four on the basis of the average flow scenario and four on the basis of the dry summer scenario. We compared so-derived *E. coli* concentrations ($PEC_{excluded}$) with the respective baseline scenarios ($PEC_{baseline}$), i.e. the average flow scenario and the dry summer scenario, as log difference ($logD$):

$$logD = \log\left(\frac{PEC_{excluded}}{PEC_{baseline}}\right) = \begin{cases} \log PEC_{excluded} - \log PEC_{baseline}, & PEC_{excluded} > 0 \\ -\infty, & \text{else} \end{cases} \tag{C.6}$$

A negative $logD$ value indicates lower concentrations in the simulation excluding the respective process. On the other hand site, positive values indicate an increase in concentration compared to the baseline scenario. $logD$ values are calculated for each river segment. If an exclusion of a process leads to predicted concentrations of 0 CFU L^{-1} $logD$ is minus infinity. Results are displayed as cumulative distribution functions in Figure C.7 and spatially resolved as maps in Figures C.8–C.9. Excluding emission processes leads lower concentrations and excluding loss processes to higher concentrations (Figure C.7).

For this analysis, we define that if the exclusion of a process leads to a deviation of less than 0.25 log units in concentration compared to the baseline scenario, the river segment is not sensitive towards the excluded process. Consequently, we call a process "sensitive" towards a river segment, if the deviation is larger than 0.25 log units. Additionally, we call a process „ very sensitive" towards a river segment, when it increases or decreases simulated concentrations by more than one order of magnitude.

Due to pumping activities in the catchment, a different proportion of cumulated flow length is affected by wastewater emissions. In the average flow scenario and the dry summer scenario 37% and 53% of cumulated flow length in the Vecht catchment are affected by WWTP emissions. The other river segments are not affected by the exclusion of WWTP emissions (see Figures C.8a and C.9a). For the average conditions scenario, this process is sensitive for 29% of cumulated flow length. For the dry summer scenario, the impact of WWTP emissions disappears faster compared to the average flow scenario (see Figures C.8a and C.9a) so that only 18% of cumulated flow length is sensitive towards

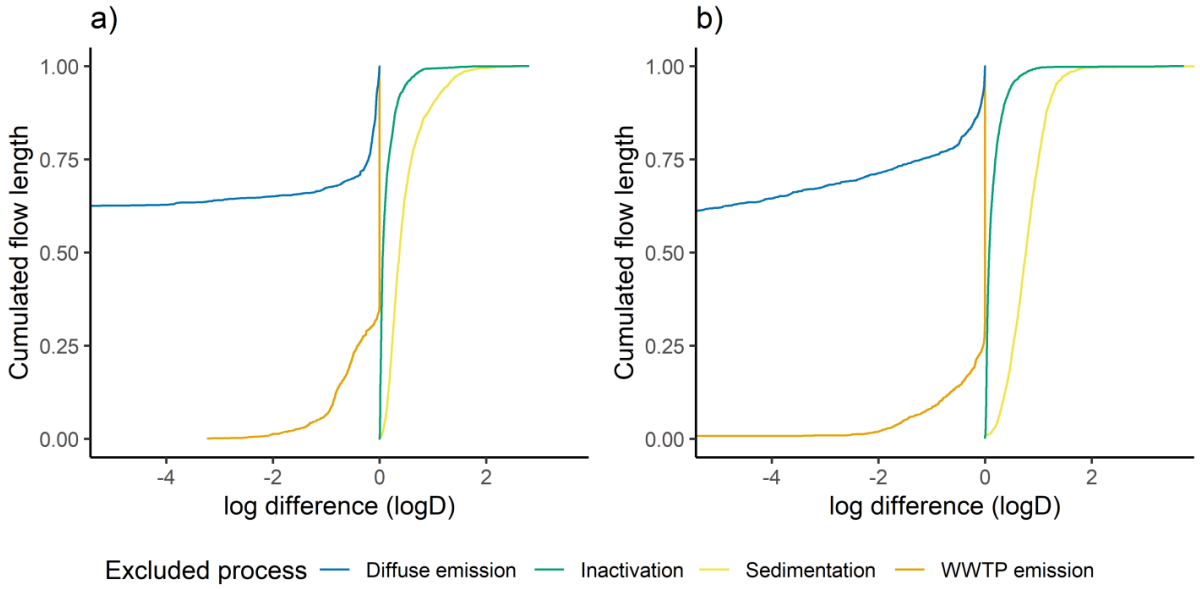

Figure C.7: Cumulated flow length of differences between simulations excluding WWTP emissions, diffuse emissions, sedimentation and inactivation and the baseline scenarios, i.e. the average flow scenario (a) and the dry summer scenario (b).

WWTP emissions. For both scenarios, less than 10% of cumulated flow lengths are very sensitive towards WWTP emissions.

Trivially, all river sections upstream of any point source are sensitive to diffuse emissions. This accounts for 63% and 47% of cumulated flow length for the average flow scenario and the dry summer scenario, respectively. Additionally, 38% and 70% of cumulated flow lengths downstream of WWTPs are sensitive and 23% and 55% are very sensitive towards diffuse emissions for the average flow scenario and the dry summer scenario, respectively. The impact of diffuse emissions is least sensitive at WWTP discharge sites (see Figures C.8 and C.9). This is where river segments are most sensitive towards WWTP emissions.

In the model, sedimentation takes place in all river segments. The process depends on the residence time of a segment, calculated by the length of the river segment and the flow velocity as well as on its depth. Both, flow velocity and depth, are generally higher for natural waterbodies in the average flow scenario. Consequently, in the average flow scenario, 72% and 10% of cumulated flow length are sensitive and very sensitive towards sedimentation, whereas in the dry summer scenario 96% and 26% of cumulated flow length are sensitive and very sensitive towards sedimentation (see Figure C.7). In canals, the flow velocity is lower compared to natural flowing waterbodies (Lämmchen et al., 2021b). Therefore, these waterbodies have a comparably longer residence time and are more sensitive towards sedimentation. In the average flow scenario, 96% and 41% of cumulated canal flow length are sensitive and very sensitive towards sedimentation. In

the dry summer scenario, flow velocity in some canals can be increased compared to the average flow scenario due to pumping activities. This results in 95% and 32% of cumulated canal flow length being sensitive and very sensitive towards sedimentation.

Just like sedimentation, inactivation is also modeled to occur catchment-wide. In contrast to sedimentation, inactivation is modeled to be independent of the depth of the respective segment. Generally, the inactivation affects concentrations less than sedimentation in both scenarios (Figures C.8 and C.9). 17% and 19% of cumulated flow length are sensitive towards inactivation, for the average flow scenario and the dry summer scenario, respectively. Less than 1% of cumulated flow length is very sensitive towards inactivation in both scenarios.

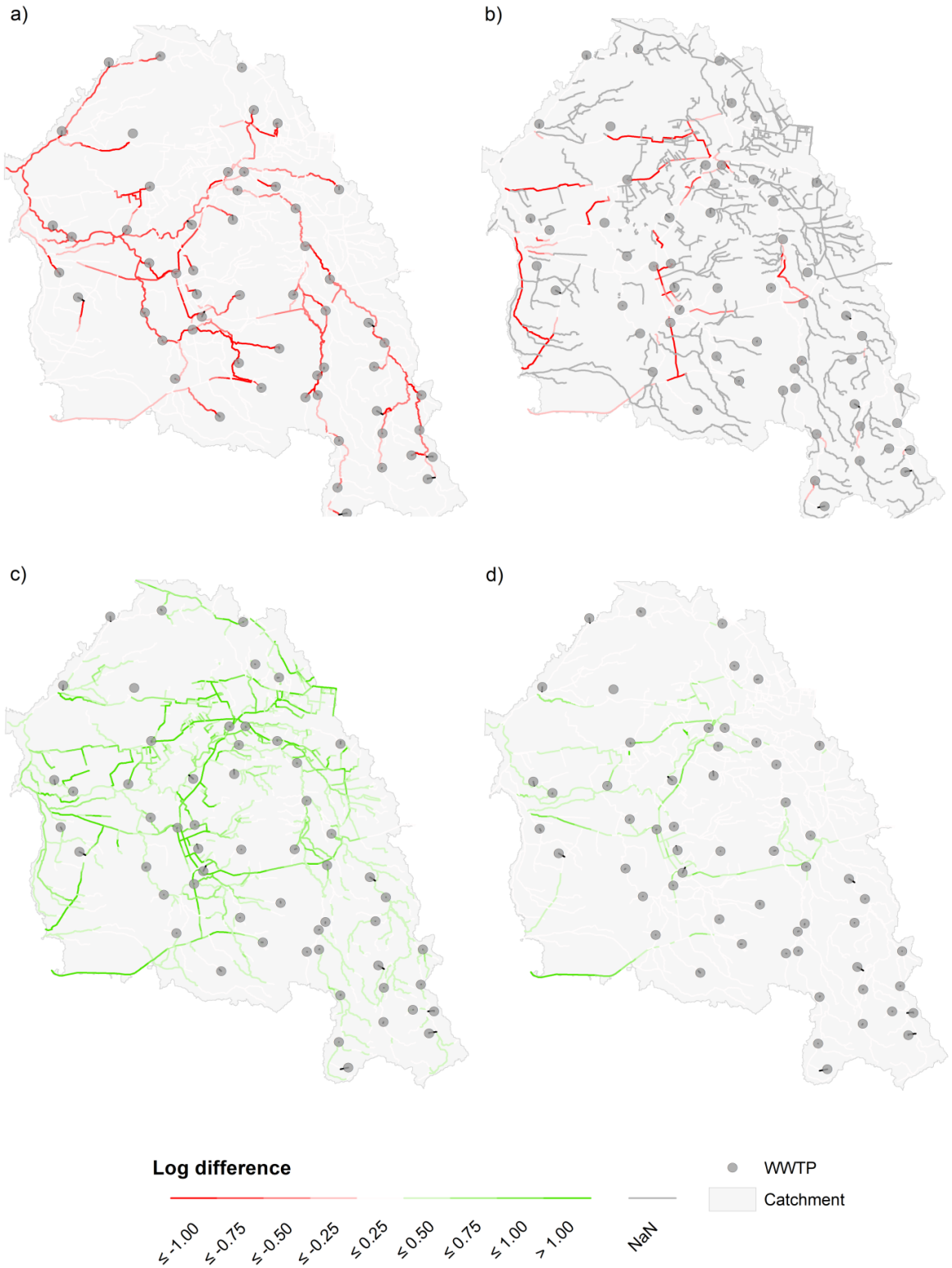

Figure C.8: Spatially resolved impact of WWTP emission (a), diffuse emission (b), sedimentation (c), and inactivation (d) for the average flow scenario. Log difference = $\log PEC_{excluded} - \log PEC_{baseline}$. $PEC_{baseline}$ and $PEC_{excluded}$ are predicted environmental concentrations in the scenarios including and excluding a process, respectively. NaN (not a number) values indicate that $PEC_{excluded} = 0$.

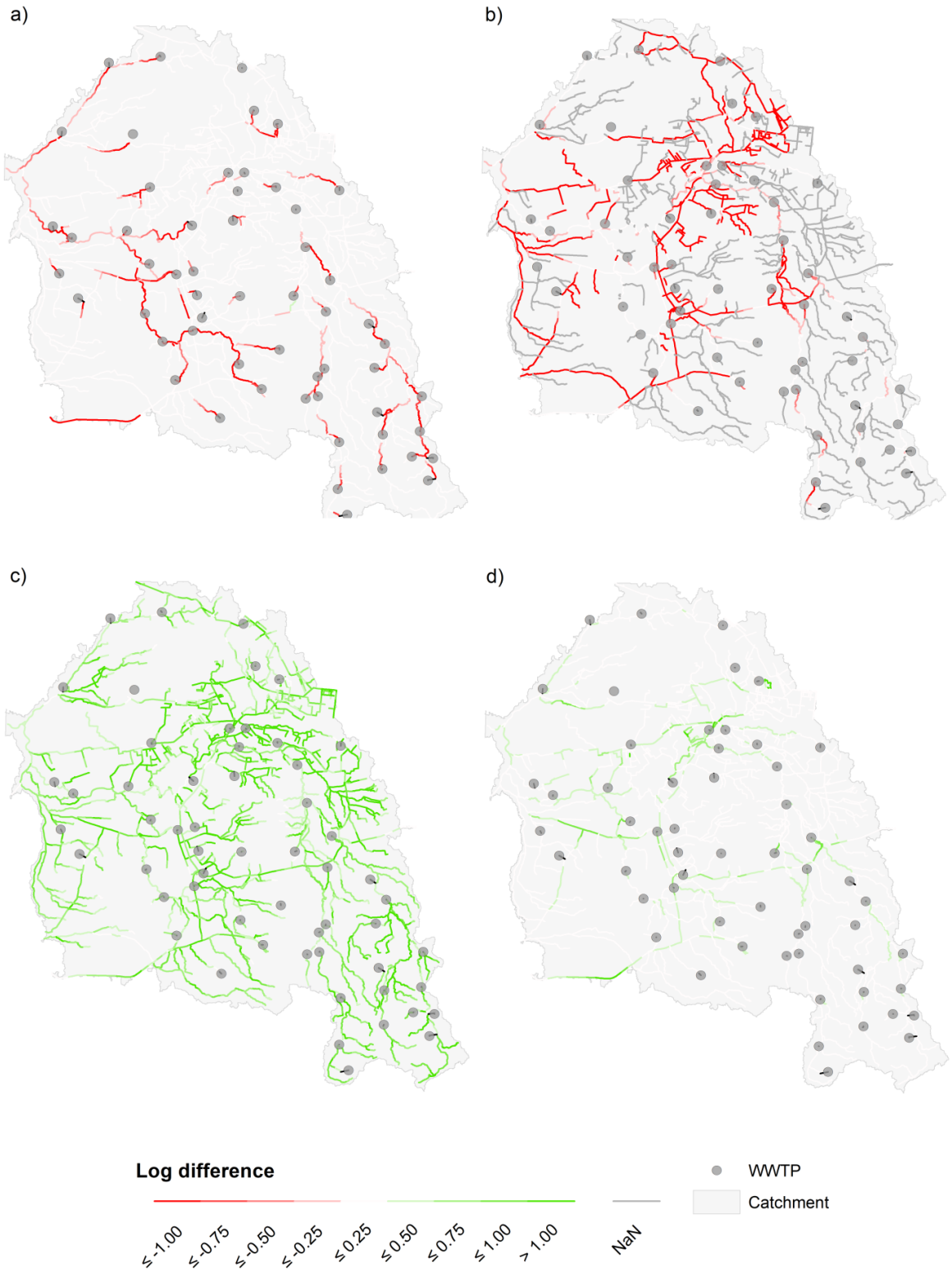

Figure C.9: Spatially resolved impact of WWTP emission (a), diffuse emission (b), sedimentation (c), and inactivation (d) for the dry summer scenario. Log difference = Log difference = $\log PEC_{excluded} - \log PEC_{baseline}$. $PEC_{baseline}$ and $PEC_{excluded}$ are predicted environmental concentrations in the scenarios including and excluding a process, respectively. NaN (not a number) values indicate that $PEC_{excluded} = 0$.

D. Appendix to Chapter 7

Table D.1: Fractions of *E. coli* attached to suspended materials in natural waterbodies van Heijnsbergen et al. (2022).

Attached fraction [%]	Source	Matrix
34.0	Jamieson et al. (2005a)	Suspended sediment
20.0	Jamieson et al. (2005a)	Suspended sediment
44.0	Jamieson et al. (2005a)	Suspended sediment
27.0	Jamieson et al. (2005a)	Suspended sediment
30.0	Garcia-Armisen and Servais (2009)	Suspended matter
36.5	Characklis et al. (2005)	Suspended solids
37.5	Characklis et al. (2005)	Suspended solids
53.6	Characklis et al. (2005)	Suspended solids
38.0	Fries et al. (2006)	Suspended particles

Table D.2: Settling velocities of suspended particles in the literature.

Settling velocity [m h^{-1}]	Source
0.1	Auer and Niehaus (1993)
0.045	Auer and Niehaus (1993)
0.36	Bai and Lung (2005)
0.058	Canale et al. (1993)
0.19	Dorner et al. (2006)
0.07	Garcia-Armisen and Servais (2009)
0.12	Jamieson et al. (2005a)
0.12	Jamieson et al. (2005a)
0.18	Jamieson et al. (2005a)
0.07	Jamieson et al. (2005a)
0.27	Jeng et al. (2005)
0.21	Liu et al. (2006)
0.1	Ouattara et al. (2011)
0.05	Wilkinson et al. (1995)

Text 1: Derivation of the relationship between the standard deviation and the percentile range of predicted concentrations.

The GREAT-ER model produces predicted concentrations in the form of lognormal distributed random variables. For Sections 7.3.1 and 7.3.2 it is helpful to have a measure to compare the range of measured or predicted lognormal distributed concentrations. This can be performed by comparison of respective σ values, i.e. the standard deviations of the underlying normal distributions. How this can be accomplished is derived and described below.

A lognormal distributed random variable X can be transformed to a normal distributed variable $Y = \ln X$ with mean μ_Y and standard deviation σ_Y. With Z being a standard normal distributed random variable with mean $\mu = 0$ and standard deviation $\sigma = 1$, it follows

$$X = e^{\mu_Y + Z \times \sigma_Y} \tag{D.1}$$

Accordingly, the p-quantile of a lognormal distributed random variable can be calculated as

$$X = e^{\mu_Y + z_p \times \sigma_Y} \tag{D.2}$$

where z_p is the p-quantile of the standard normal distribution, i.e. $\Phi(z_p) = p$, where Φ is the distribution function of the standard normal distribution.

The required standard deviation σ_Y of the underlying normal distribution can be calculated from the mean μ_X and the standard deviation σ_X of the lognormal distributed variable:

$$\sigma_Y = \sqrt{\ln\left(\frac{{\mu_X}^2 + {\sigma_X}^2}{{\mu_X}^2}\right)} \tag{D.3}$$

The $(1 - \alpha)$ percentile range $[X_{\alpha/2}, X_{1-\alpha/2}]$ can be written as

$$\left[e^{\mu_Y + z_{\alpha/2} \times \sigma_Y}, e^{\mu_Y + z_{1-\alpha/2} \times \sigma_Y}\right] \tag{D.4}$$

Then, the ratio of the two boundary values of the $(1-\alpha)$ percentile range is

$$\frac{X_{1-\alpha/2}}{X_{\alpha/2}} = \frac{e^{\mu_Y + z_{1-\alpha/2} \times \sigma_Y}}{e^{\mu_Y + z_{\alpha/2}\sigma_Y}} \tag{D.5a}$$

$$= e^{\sigma_Y \times (z_{1-\alpha/2} - z_{\alpha/2})} \tag{D.5b}$$

$$= e^{\sigma_Y \times 2 \times z_{1-\alpha/2}} \tag{D.5c}$$

where we use the fact that $z_{1-\alpha/2} = -z_{\alpha/2}$.

Drawing the decadic logarithm of the ratio yields a linear relationship between the standard deviation σ_Y and the number of orders of magnitude encompassed by the $(1-\alpha)$ percentile range:

$$\log\left(e^{\sigma_Y \times 2 \times z_{1-\alpha/2}}\right) = \frac{\ln e^{\sigma_Y \times 2 \times z_{1-\alpha/2}}}{\ln 10} \tag{D.6a}$$

$$= \frac{\sigma_Y \times 2 \times z_{1-\alpha/2}}{\ln 10} \tag{D.6b}$$

$$= 0.87 \times \sigma_Y \times z_{1-\alpha/2} \tag{D.6c}$$

In summary:

$$\log\left(\frac{X_{1-\alpha/2}}{X_{\alpha/2}}\right) = 0.87 \times \sigma_Y \times z_{1-\alpha/2} \tag{D.7}$$

Thus, the logarithm of the percentile range linearly depends on the standard deviation σ_Y. Formula D.7 then allows for simple interpretation of the distribution width on the log scale. As an example: for a lognormal distributed random variable with $\sigma_Y = 1.80$, the 80% percentile range ($\alpha = 0.2$, $z_{1-\alpha/2} = 1.28$) covers 2 orders of magnitude (factor 100):

$$\log\left(\frac{X_{P90}}{X_{P10}}\right) = 0.87 \times 1.80 \times 1.28 = 2 \Rightarrow r_{80\%} = \frac{X_{P90}}{X_{P10}} = 100 \tag{D.8}$$

Example 1: Comparison of predicted and measured concentration ranges:

For a lognormal distributed predicted concentration let $\sigma_{pred} = 1.77$ be the standard deviation of the underlying normal distribution. Corresponding measured concentrations led to lognormal distributed concentrations with a standard deviation σ_{meas} of 1.50 of the underlying normal distribution. With equation D.6a, the 80% percentile range $r_{80\%}$ ($\alpha = 0.1$, $z_{1-\alpha/2} = 1.28$) of predicted and measured concentrations then covers a range of factor 94 and 47, respectively. In this case, the 80% percentile range of predicted

concentrations is two times larger compared to the range of measured concentrations. From equation D.7 it follows that we can calculate this factor directly as follows:

$$\frac{r_{(1-\alpha)P,pred}}{r_{(1-\alpha)P,meas}} = \frac{10^{0.87\times\sigma_{pred}\times z_{1-\alpha/2}}}{10^{0.87\times\sigma_{meas}\times z_{1-\alpha/2}}} = 10^{0.87\times z_{1-\alpha/2}\times(\sigma_{pred}-\sigma_{meas})} \tag{D.9}$$

With $\sigma_{pred} - \sigma_{meas} = \Delta\sigma = 0.27$ we obtain $10^{0.87\times1.28\times0.27} \approx 2$.

Thus, the 80% percentile range of predicted concentrations is two times larger compared to the 80% percentile range of measured concentrations. For the 90% percentile range, a difference of factor 2 is obtained if $\Delta\sigma = 0.21$ and for the 95% percentile range if $\Delta\sigma = 0.18$ (see Table D.3).

Example 2: Sensitivity of model parameters on the width of the predicted concentration distribution:

In the sensitivity analysis, results of the reference scenario, where all variable parameters are defined as probability distribution, are compared to a test scenario. In the test scenario, the parameter, whose sensitivity on the width of the predicted concentration distribution shall be investigated, is kept constant. We can calculate the factor of reduction of the distribution width using equation D.9 accordingly.

For a selected waterbody, the standard deviation in the reference scenario was $\sigma_{ref} = 1.90$, while in the test scenario it was significantly lower with $\sigma_{test} = 1.0$. We can now calculate the factor of reduction of the distribution width using Equation D.9 accordingly. Inserting $\Delta\sigma = \sigma_{ref} - \sigma_{test} = 0.9$ into equation D.9 leads to a change of the 80% percentile range $r_{80\%}$ ($\alpha = 0.2$, $z_{1-\alpha/2} = 1.28$) of a factor of ten ($10^{0.87\times0.9\times1.28}$). In this case, the uncertainty and/or variability of the selected parameter was responsible for a large fraction of the concentration variability in the model. Table D.3 shows the spread for different percentile ranges and the required difference in standard deviation to reduce the spread by factor two or ten, respectively.

Table D.3: Effect of changing sigma on percentile range of predicted concentrations.

Percentile range	spread of percentile (log scale)	S.d. difference for factor two spread reduction	S.d. difference for factor ten spread reduction
$r_{80\%}$	$0.87 \times 1.28 \times \sigma_Y$	$\Delta\sigma_Y = 0.27$	$\Delta\sigma_Y = 0.90$
$r_{90\%}$	$0.87 \times 1.64 \times \sigma_Y$	$\Delta\sigma_Y = 0.21$	$\Delta\sigma_Y = 0.70$
$r_{95\%}$	$0.87 \times 1.97 \times \sigma_Y$	$\Delta\sigma_Y = 0.18$	$\Delta\sigma_Y = 0.59$

Acknowledgements

There are very many people without whom I would not have been able to write this thesis, not to speak of finalizing this work. I cannot and will not mention all of them, because a comprehensive list of people to whom I am indebted could never claim to be complete. I would like to explicitly thank some of them at this point.

First of all, I would like to sincerely thank my supervisor Dr. Jörg Klasmeier, who already supervised both my bachelor's and master's thesis, significantly influenced my interest as well as my professional orientation towards environmental exposure modeling and offered me the opportunity of writing my PhD thesis under his supervision. I am very grateful for this opportunity. During the PhD period, Jörg supported me with his expertise and continuous advisory exchange. Likewise, I would like to thank my second supervisor Professor Heike Schmitt, without whom I would never have gained such deep insights into the exposure and environmental dynamics of bacteria. A big thank you also to Dr. Jürgen Berlekamp for his technical support in all GIS matters.

I am indebted in particular to the MEDUWA project management team, which accompanied the whole project, created platforms for exchange and established important contacts. A special thanks goes to Alfons Uijtewaal, whose countless efforts made the MEDUWA project possible in the first place.

The entire Applied Systems Science research group deserves a special mention for the professional exchange, support, and warm fellowship. It cannot be taken for granted that all colleagues always have an ever open door for concerns and problems. I really enjoyed writing my thesis in a constructive, helpful and familiar environment. At this point I would like to thank Elke, Claudia and Nina in particular for their administrative support.

I am especially grateful and thankful to my (MEDUWA) PhD colleagues. The common experience of the PhD and project time, the sharing of experiences and the common exchange made this time much easier - the one or other beer, playing "Wickinger Schach", "Achtung die Kurve" or "Backgammon" have certainly contributed to this.

I am deeply indebted to my family and friends. My family has supported me my entire life from school through university to doctorate and of course in all the important aspects of life. I cannot be grateful enough. My girlfriend Franzi has always supported me, lifted me

up, endured my frustrations and was my strongest support, especially in the final phase of my doctorate. I would especially like to thank my friends from Osnabrück - in particular "Osnabrück Oldschool" - who balanced all the stress with lots of humor, activities and vacations and have always given me motivation and strength.

Erklärung über die Eigenständigkeit der erbrachten wissenschaftlichen Leistung

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Aufgrund der Zusammenarbeit mit Kollegen bei der Konzeptentwicklung und der Ausarbeitung der Kapitel 4–7 dieser Arbeit als Publikationen wurde an vielen Stellen die 'Wir'-Form verwendet. Die Aufschlüsselung der Beteiligung der Co-Autor:innen an den jeweiligen Publikationen ist in Kapitel 3 erfolgt.

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