ACES REPORT 15

Ciprofloxacin

EQS data overview

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EQS DATA OVERVIEW

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Preface

The Department of Environmental Science and Analytical Chemistry (ACES) at Stockholm University was commissioned, by the Swedish Agency for Marine and Water Management and the Swedish Environmental Protection Agency, to perform a literature overview and possible EQS derivation for the specific pollutant ciprofloxacin. The work was performed under the Water Framework Directive (2000/60/EC) using the European Communities's guidance document "Technical Guidance for Deriving Environmental Quality Standards".

The report was prepared by Sara Sahlin and Marlene Ågerstrand at ACES, in collaboration with Joakim Larsson at the Department of Infectious Diseases, Institute of Biomedicine, and Centre for Antibiotic Resistance Research at University of Gothenburg.

Stockholm, April 23rd, 2018
The Department of Environmental Science and Analytical Chemistry (ACES)
Stockholm University

Förtydligande från Havs- och vattenmyndigheten

Havs- och vattenmyndigheten planerar att ta med ciprofloxacin bland de ämnen som regleras i Havs- och vattenmyndighetens föreskrifter (HVMFS 2013:19) om klassificering och miljökvalitetsnormer avseende ytvatten[1]. Stockholms Universitet har därför på uppdrag av Havs- och vattenmyndigheten och Naturvårdsverket tagit fram beslutsunderlag för att kunna etablera bedömningsgrunder för ciprofloxacin. I rapporten som sammanställts i samarbete med Göteborgs universitet har flera alternativa värden tagits fram. Utifrån litteratursökning och granskning av underlag har förslag på värden beräknats utifrån de riktlinjer som ges i CIS 27 (European Communities, 2011) för konventionell härledning, men också baserats på fördelning av MIC värden för heterotrofa bakterier och direkta selektionsstudier med beaktandet av risk för resistensutveckling. Slutgiltigt val av värde att utgå ifrån vid statusklassificering har föreslagits av Havs- och vattenmyndigheten efter dialog med deltagare i en arbetsgrupp (representanter från Kemikalieinspektionen, Naturvårdsverket och Läkemedelsverket).

Utifrån den konventionella metodiken (CIS 27) men efter att ha uteslutit data för heterotrofa bakterier beräknades årsmedelvärет till 0,5 µg/l respektive 0,05 µg/l för limniska respektive marina vatten. Beaktar man istället resistensutveckling hamnar värdet på 0,1 µg/l, dvs. mellan värdena för limnisk och marin vattenfas. Då det inte finns något som tyder på att marina organismer är mer känsliga än limniska föreslås värdet **0,1 µg/L** men uttryckt som **maximal tillåten vattenkoncentration** då det i princip bara krävs ett selektionstryck under en kortare tid för att resistens ska uppstå. Dessutom är de två vattenvärdena som avser ”kronisk toxicitet” styrda av toxicitet mot organismer med en väldigt kort generationstid vilket också motiverar detta. Förslag på värde bedöms därför skydda både pelagiska organismer (inklusive blågröna alger och heterotrofa bakteriers nedbrytande funktion) och oss människor indirekt.

Notera att bedömningsgrunder för ciprofloxacin ännu inte har beslutats.

[1] https://www.havochvatten.se/hav/vagledning--lagar/foreskrifter/register-vattenforvaltning/klassificering-och-miljokvalitetsnormer-avseende-ytvatten-hvmfs-201319.html

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1. METHODS CONSIDERATIONS

Switzerland established EQS values for ciprofloxacin in 2013 (Swiss Ecotox Centre, 2013). The EQS derivation in the current report is based on the data from from the Swiss Ecotox Centre, in combination with ecotoxicity studies published in the peer-reviewed literature after 2013. In addition, a literature search was also performed for studies containing data on sediment and mammal toxicity, as well as antibiotic resistance selection. The ecotoxicity studies used in the report from the Swiss Ecotox Centre were evaluated for their reliability and relevance by the Swiss Ecotox Centre.

The following databases were used when searching for studies: Web of Science, Scopus, ETOX, Ekotoxzentrum, UBA, IRIS, RIVM, OECD, and WikiPharma. The following keywords were used: Ciprofloxacin* ecotoxicity, aquatic toxicity, toxicity, sediment toxicity, MIC, MSC, resistance, EC50, LC50, EC10, NOEC, rodents, avian, mammals, bioaccumulation, BAF, bioconcentration, bioavailability. The literature search was conducted in mid 2017. The database "Pharmaceuticals in the environment", available from the German Environmental Protection Agency (UBA), was used to collect measured environmental concentrations. Additionally, the European Committee on Antimicrobial Susceptibility Testing database (EUCAST: http:// www.eucast.org) was used to collect Minimal Inhibitory Concentration (MIC) values for a large range of bacterial species and strains.

1.1 EQS derivation

EQS derivations aim to protect identified receptors of risks such pelagic and benthic ecosystems and human health. In line with the European Communities (2011), Quality Standards (QS) are derived for pelagic communities to cover long-term (Annual Average: AA-QS) and short-term (Maximum Acceptable Concentration: MAC-QS) exposure. Risks for benthic communities and secondary poisoning for pelagic biota and top predators are addressed in $QS_{sediment}$ and $QS_{biota\ sec\ pois}$, respectively. However, in the case of antibiotics, an additional risk has been identified: the potential of selecting for antibiotic resistance in the environment, with potential consequences for human health and domestic animals (Ashbolt et al, 2013). Therefore, two different types of QS values for surface water were calculated:

1) Conventional QS_{pelag} values, excluding bacteria (except cyanobacteria since it is considered to have the same status as algae according to European Communities, 2011) and basing QS on species conventionally used for ecotoxicity testing (section 10.1).

2) QS_R values for risk of antibiotic resistance selection. This was based both on experimental derivation of Minimal Selective Concentrations in *E. coli* (Gullberg et al., 2011), empirical LOEC and NOEC values for resistance selection in complex aquatic biofilms (Kraupner et al., 2018) supported further by the distribution of MIC data across bacterial species and strains according to the approach suggested by Bengtsson-Palme and Larsson, 2016) (section 10.2).

1.2.1 Protection of pelagic ecosystems (MAC and AA-QS)

Antibiotics entering ecosystems in high enough concentrations may alter the microbial community structure and inhibit or promote ecological functions, such as nutrient regeneration, organic matter mineralization, and pollutant degradation (Ding and He, 2010; Näslund et al., 2008). During the preparation of this report, questions were raised regarding the use of bacterial species as a protection goal. Because antibiotics are designed to target bacteria it could be considered relevant to

study effects on target organism in addition to conventional environmental risk assessment species such as algae, crustaceans or fish. On the other hand, the main concern with regards to bacteria is whether the functionality of exposed microbial communities will be altered, and not so much if certain species or strains are favoured or disfavoured. Linked to the latter is of course that selection for resistance seems plausible if exposure levels are high enough to reduce growth of some species, even if there is no net effect on the functionality of the communities. In fact, from an ecological point of view, resistance development contributes to resilience (a good thing) of the community, while it poses an increased risk for humans.

The EQS derivation was based on (eco)toxicity studies for ciprofloxacin, ciprofloxacin hydrochloride (C-HCl) and ciprofloxacin hydrochloride hydrate (C-HCl-H2O). The molar ratio was used to convert C-HCl and C-HCl-H2O to ciprofloxacin (C-HCl to ciprofloxacin = 0.9; C-HCl-H2O to ciprofloxacin= 0.859 (Swiss Ecotox Centre, 2013). The derivation for protection of pelagic ecosystems and secondary poisoning was performed under the Water Framework Directive (2000/60/EC) using the European Communities's (2011) guidance document "Technical Guidance for Derivning Environmental Quality Standards", using conventional ecotoxicity testing species.

1.2.2 Potential for selection of antibiotic resistance (QS_R)

The European Communities (2011) does not stipulate details regarding potential for selection of antibiotic resistance. Assessing and understanding human health risks associated with antibiotic pollution is a complex task that involves many steps (Ashbolt et al., 2013). Because of the different possible risk scenarios and pathways involved, a generalized, quantitative risk assessment has not been considered feasible. Nevertheless, a selection pressure from antibiotics in the environment, favouring resistant bacteria over sensitive ones, is considered a risk and a critical component in these scenarios (Lupo et al., 2012; European Commission, 2017; Bengtsson-Palme and Larsson, 2018). Therefore, the basis for developing QS data with regards to resistance, here called $QS_{R,}$ is that the antibiotic in question should not select for resistant bacteria in the external environment.

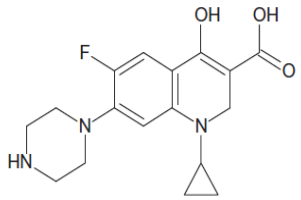
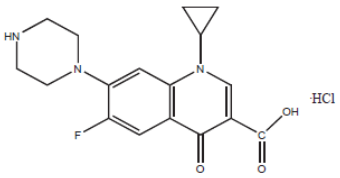
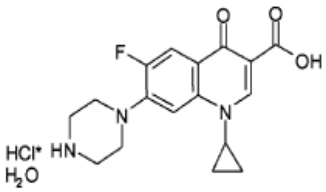
Minimum Selective Concentrations (MSCs) define the minimum concentration of an antibiotic that is predicted to select for resistance in a given situation. It should be noted that the MSC is neither a NOEC nor a LOEC. It is rather the predicted lowest effect concentration and at the same time the predicted highest no effect concentration. The MSC concept is therefore slightly different than the more classical LOEC/NOEC concept commonly applied in (regulatory) ecotoxicology. Comparing the growth of a resistant strain over a sensitive wild-type strain growing in the same test tube over many generations can be the basis for such assessments (Gullberg et al., 2011). As the MSC is dependent on the costs of carrying the resistance factor, a derived MSC will depend on the resistance factor studied, its larger context, and the presence of compensatory mutations. Hence, in practice, only a subset of resistance factors and context can be tested. This strategy of deriving an MSC based on growth competition between two strains in the lab is very sensitive but may not fully reflect the complex interactions and competition situations that occur in microbial ecosystems. Alternative ways to derive MSCs based on resistance selection in complex communities have therefore been proposed (Lundström et al., 2016; Kraupner et al., 2018). If several reliable and applicable empirical MSCs are available, it is therefore, from a regulatory point of view, advisable to choose the lowest MSC.

Selective concentrations may also be theoretically estimated based on MIC (Bengtsson-Palme and Larsson, 2016). The MIC values refer to the concentration that completely inhibits growth of a strain, and provide by itself limited information about selective concentration. However, it is reasonable to

assume that a concentration that completely inhibits growth of some strains would also provide a selective advantage for resistant strains of that species. Based on this assumption, the lowest available MIC constitutes the upper-boundary of a predicted MSC.

Bengtsson-Palme and Larsson (2016) used MIC data from the EUCAST database to identify the lowest available MIC for over 100 antibiotics. The lowest MIC were size adjusted (extrapolated through modeling) for the number of tested species available. For ciprofloxacin, one of the most investigated antibiotics, there were MIC data for 70 bacterial species and over 300,000 different isolates. A $PNEC_R$ (Predicted No-Effect Concentration for Resistance selection) was estimated based on the size-adjusted MIC by applying an assessment factor (AF) to take into account that the MSC is predicted to be lower than the lowest MIC. How much lower is, however, difficult to know. Bengtsson-Palme and Larsson (2016) applied an assessment factor of 10.

2. CHEMICAL IDENTITY[1]

Common name	Ciprofloxacin
Chemical name (IUPAC)	1-Cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydro-3-quinolinecarboxylic acid
Synonym(s)	3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)
Chemical class	Carboxyl-Fluoroquinolone
CAS number	C: 85721-33-1 C-HCl: 93107-08-5 C-HCl-H2O: 86393-32-0
EU number	C: 617-751-0 C-HCl: na C-HCl-H2O: 617-845-1
Molecular formula	C: $C_{17}H_{18}FN_3O_3$ C-HCl: $C_{17}H_{18}FN_3O_3HCl$ C-HCl-H2O: $C_{17}H_{18}FN_3O_3HCl$-H2O
Molecular structure	C:  C-HII:  C-HCl-H2O: 
Molecular weight (g.mol^{-1})	C: 331.35 C-HCl: 367.9 C-HCl-H2O: 385.8

1 = Data collected from Swiss Ecotox Centre (2013).

3. EXISTING EVALUATIONS AND REGULATORY INFORMATION

Annex III EQS Dir. (2008/105/EC) amended by Directive 2013/39/E	Not included. Has been proposed as candidate but did not fulfil all selection criteria.
Existing Substances Reg. (793/93/EC)	Not applicable
Pesticides (91/414/EEC)	Not included in Annex I
Biocides (98/8/EC)	Not included in Annex I
PBT substances	Not investigated
Substances of Very High Concern (1907/2006/EC)	No
POPs (Stockholm convention)	No
Human and veterinary environmental risk assessment (EMEA/CHMP/SWP/4447/00; CVMP/VICH/592/1998; CVMP/VICH/790/2003)	No information available
NORMAN List of Emerging substances	Included
Voluntary environmental classification at fass.se	- Use of ciprofloxacin has been considered to result in moderate environmental risk. - Not ready biodegradable. - Low potential for bioaccumulation.
Environmental hazard and risk classification at janusinfo.se	- Toxic to aquatic organisms. - Ability to resist degradation in the aquatic environment. - No ability for accumulation in adipose tissue of aquatic organisms. - Moderate environmental risk.
Endocrine disrupter	Not investigated
REACH Annex III	- Suspected carcinogen (genotoxic and non-genotoxic). - Suspected mutagenic. - Suspected persistent in the environment. - Suspected toxic for reproduction.
CLP	- H400: Aquatic acute 1 (very toxic to aquatic life). - H410: Aquatic chronic 1 (very toxic to aquatic life with long lasting effects). - H412: Aquatic chronic 3 (harmful to aquatic life with long lasting effects). - H361: Reproduction 2 (suspected of damaging fertility or unborn child). - H315: Skin Irriti. 2 (cause skin irritation). - H317: Skin Sens. 1 (may cause allergic skin reaction). - H319: Eye Irrit. 2 (causes serious eye irritation). - H334: Resp. Sens. 1 (may cause allergy, asthma or breathing difficulties if inhaled). - H335: STOT SE (may cause respiratory irritation).

4. PROPOSED QUALITY STANDARDS (QS)

4.1 Environmental Quality Standard (EQS)

QS for potential of resistance development is the "critical QS" for derivation of an Environmental Quality Standard.

	Unit	Value	Comments
Proposed MAC-EQS$_R$ [antibiotic resistance]	[µg.L^{-1}]	0.1	Critical QS See section 10.2.2
Proposed AA-QS for [conventional pelagic QS]	[µg.L^{-1}]	0.5	See section 10.1.3
Proposed MAC-QS for [conventional pelagic QS]	[µg.L^{-1}]	3.6	See section 10.1.1
Proposed QS$_{sediment}$	Not derived		See section 8.3
Proposed QS$_{biota\ sec\ pois}$	[µg.kg^{-1}$_{biota\ ww}$]	833	See section 11.1

5. MAJOR USES AND ENVIRONMENTAL EMISSIONS

5.1 Summary of Uses and Quantities

Ciprofloxacin is a second-generation of fluoroquinolone antibiotic, exhibiting a broad spectrum of activity against aerobic gram-negative and gram-positive bacteria (Ebert et al., 2008; Nie et al., 2008; Fisher et al., 1988). Ciprofloxacin is worldwide used in human medical treatment as well as veterinary medical treatment and aquaculture (Nie et al., 2008). Ciprofloxacin is used to treat human infections in the urinary tract, respiratory system, gastrointestinal system, and abdomen (Iigin et al., 2015).
The yearly consumption of ciprofloxacin in Sweden was estimated to 1.104 mg/capita/day compared to the mean value for the European use of 0.652 mg/capita/day (Johnson et al., 2015). In 2012, ciprofloxacin accounted for 71% of the consumption of second-generations quinolones in EU (ECDC, 2014).

Ciprofloxacin is not included in the list of veterinary antibiotic for sales within EU (EMA, 2014). However, ciprofloxacin is an active metabolite of the veterinary antibiotic enrofloxacin (Idowu et al., 2010; Tyczkowska et al., 1989). For example, 50% of the administrated enrofloxacin was transformed to ciprofloxacin in cows (Lykkberg et al., 2007). Consequently, ciprofloxacin concentrations in surface water may originate from both human and veterinary use (Knapp et al., 2005). In Sweden enrofloxacin is intended for use of treatment in livestock (cattles) (Fass.se, n.d.1). In 2016, 33 000 ml (solution for injection 100 mg/ml) of enrofloxacin was prescribed in Sweden according to N-vet Läkemedel (email correspondence February 6, 2017). The EU regulation of veterinary use of pharmacologically active substances specifies the maximum residue limits in foodstuffs of animal origins of enrofloxacin and its metabolite ciprofloxacin to 100-300 µg/kg (European Communities, 2010).

5.2 Environmental Emissions

Antibiotics are released to the environment via effluents from wastewater treatment plants (WWTPs), hospital wastewater, processing plant effluents, waste from manufacturing of pharmaceuticals, land applications of human and agricultural waste, landfills leakage, and aquaculture (Halling-Sørensen et al., 2000; Kümmerer, 2009; Sarmah et al., 2006; Larsson, 2014a).

Ciprofloxacin orally administered to humans are primarily excreted unmetabolized with an estimated 44.7% of the excreted dose in urine and 25% in faeces (Fass.se, n.d.2). The mean elimination of ciprofloxacin from water in five Swedish sewage treatment plants (STP) was estimated to 87% in a study by Lindberg et al. (2005). Similar results have been reported for other conventional WWTPs (Watkinson et al., 2007; Batt et al., 2007). Lindberg et al. (2014) estimated the removal efficiencies (based on measurements of influent and effluent samples) of ciprofloxacin from sewage water to 58% in the STP of Umeå city. In the same study the average mass flow was estimated to 4681 and 1939 mg/day for influent and effluent, respectively. Another study found that approximately 3.6% of the total mass flow of ciprofloxacin in raw sewage was found in the final effluent and 77% in digested sludge when analysing samples of raw sewage water, particles, effluents, and sludge in Umeå STP (Lindberg et al., 2006). The digestion efficiency (reduction in the sewage's organic content) was 42% and ciprofloxacin was assessed as relatively resistant to digestion (Lindberg et al., 2006). Worldwide measurements of effluents discharges range from ng/L to several mg/L, the latter from pharmaceutical manufacture. Some of the measurements found in the scientific literature from inlet, effluent, and sludge of WWTPs/STPs are presented in table 3.

6. ENVIRONMENTAL BEHAVIOUR

6.1 Environmental distribution

The ciprofloxacin molecule includes a carboxylic acid group (pK_{a1}=6.1) and an amine group in the piperazine moiety (pK_{a2}=8.7) both affecting the pH-dependent behaviour on solubility and hydrophobicity (results reported in Gu et al., 2005). At neutral pH ciprofloxacin carries both a negative and positive charge, it is a neutral compound despite the charges within the molecule (Kümmerer et al., 2008). Physicochemical properties of ciprofloxacin are summarized in table 1.

Ciprofloxacin strongly sorbs to organic suspended particles in water, sludge and sediments (Cardoza et al., 2005; Lindberg et al., 2005; Golet et al., 2003). Códova-Kreylos and Scow (2007) concluded that the sorption to sediments (salt marshes) was positively correlated with clay content and negatively correlated with pH. Previous studies demonstrate a pH dependent sorption of ciprofloxacin onto aluminosilicate, aluminium oxides, amorphous iron oxides, goethite, and soils and soil minerals. The sorption to soils and soils mineral occur via sorption onto aluminosilicate clays via cation exchange, cation bridging, or via surface complexation (Pei et al., 2010).

Ciprofloxacin adsorbed by sediment is believed to be less bioavailable, e.g. Códova-Kreylos and Scow (2007) showed that the modifying effect on microbial communities was lower in sediment with greater sorption potential. This can also be argued from the levels often found in sludge (mg/kg) (Lindberg et al., 2006) which if translated to mass per volume would be a lethal concentration for a wide variety of bacteria (EUCAST database). But, based on observations, sludge does not appear to have a particularly high proportion of ciprofloxacin resistant bacteria (Reinthaler et al., 2003).

Potential of bioaccumulation in lakes has been studied in rivers and lakes in China (Xie et al., 2017; Goa et al., 2012; Bai et al., 2014), with highest BAF reported for fish (545-3262 L/Kg). Goa et al. (2012) reported tissue specific (muscle) BAF of 3262 based on dry weight, however, BAF should preferably be expressed as whole-body concentrations and based on wet weight (Arnot and Gobas, 2006; European Communities 2011). Also, some of the fish samples seem to be collected during a different time period than the water samples. Likewise, Xie et al. (2017) reported tissue specific BAFs for fish rather than whole body. The BAF increased with increasing trophic levels (except for shrimp), but no biomagnification could be identified in the food web (TMF<1) (Xie et al., 2017).

Table 1. Physicochemical properties of ciprofloxacin.

		Reference
Water solubility (mg.L^{-1})	**C:** 30 000 (exp; 20°C); 11 500 (est, 25°C)	EPI Suite, 2011[1]
	C-HCl-H_2O: 30 000 (exp; 20°C); 38 400 (exp; 30°C)	Varanda et al., 2006[1]
	C: 292 (pH 5); 59 (pH 7); 200 (pH 9) (exp.)	Gagliano and McNamara, 1996 (Bayer Report No. 106436)[1]
Volatilisation		
Vapour pressure (Pa)	**C:** < $1.33 \cdot 10^{-5}$ (exp; 25°C)	Gagliano and McNamara McNamara, 1996 (Bayer Report No. 106436)[1]
	C: $3.8 \cdot 10^{-11}$ (est.)	EPI Suite, 2011[1]
Henry's Law constant (Pa.m3.mol^{-1})	$5.16 \cdot 10^{-14}$ (est; 25°C)	EPI Suite, 2011[1]
Adsorption		
Organic carbon – water partition coefficient (Log K_{OC})	**C:** 4.55; 4.62; 4.68; 5.13 (exp. different soils)	Gagliano and McNamara, 1996 (Bayer Report No. 106556)[1]
	C: ca. 4.3 (exp; pH 7.3 and 7.8)	Cardoza et al., 2005[1]
	C: 4.8 (soil)	Tolls, 2001
	C: 4.5 -5.8 (exp. salt march sediments)	Córdova-Kreylos & Scow, 2007
Partition coefficient (Log $K_{d\text{- solid-water}}$)	4.15 (sediment)	Goa et al., 2012
	4.3 (sludge, pH 7.5–8.4) 2.6 (soil, pH 5)	Golet et al., 2003
Suspended matter – water partition coefficient ($K_{suspwater}$)	Not investigated	
Bioaccumulation		
Octanol-water partition coefficient (Log K_{ow})	**C:** 0.28 (exp.)	Takacs-Novak et al., 1992[1]
	C: -1.07 (pH 5); -0.783 (pH 7); -1.44 (pH 9) (exp.)	Gagliano and McNamara, 1996 (Bayer Report No. 106436)[1]
BCF (measured)	Not found	
BCF (estimated, L. kg^{-1} ww)	3.162	EPI Suite (BCFWIN v2.17) (in Ortiz et al., 2013)
BAF (field, L. kg^{-1})	138 (Phytoplankton ww)	Xie et al., 2017
	254 (zooplankton ww)	
	504 (zoobenthos ww)	
	197 (shrimp ww)	
	150 (crab dw)	Bai et al., 2014
	3262 (fish, muscle dw)	Goa et al., 2012
	545 (fish, muscle ww)	Xie et al., 2017
	811 (fish, gills ww)	
	1210 (fish, brain ww)	
	2008 (fish, liver ww)	
BAF (estimated, L. kg^{-1})	0.98	EPI Suite (in Ortiz et al., 2017)
BSAF (zoobenthos)	0.032	Xie et al., 2017
TMF	<1	

1 = Data collected from Swiss Ecotox Centre (2013). exp = experimentally. est= estimated.

6.2 Abiotic and Biotic degradations

Ciprofloxacin was reported as not readily biodegradable (Kümmerer et al., 2000; Al-Ahmad et al., 1999; Girardi et al., 2011), and Girardi et al. (2011) suggest slow degradation in soils with 0.9% of ciprofloxacin being mineralized after 93 days (table 2). Ciprofloxacin can undergo photolysis degradation with reported half-times from a few minutes to weeks, depending on light intensity and spectrum (Toolaram et al., 2016; Cardozoa et al., 2005; Babić et al., 2013; Lin et al., 2010, table 2). Toolaram et al. (2016) identified nine transformations products as a result of UV photolysis. The transformation products appear to retain the ring core of the quinolone structure, suggested being essential for antibacterial activity (Paul et al., 2010; Toolaram et al., 2016). Ecotoxicity test performed with high light intensity may result in photolysis degradation of ciprofloxacin, and consequently the concentrations may not be reliable if not analytically confirmed.

Table 2. Abiotic and biotic degradation of ciprofloxacin.

		Master reference
Hydrolysis	No hydrolysis for 5 days (exp, pH 5, 7 and 9 at 50 °C)	Gagliano and McNamara, 1996 (Bayer Report Nr. 106430)[1]
Photolysis (DT_{50})	**C: 46 hours** (artificial light (470 µE m-2 s-1), 250 µg/L, pH 7.5-8.6, 20 ° C) 1**.9 hours** (simulated Sunlight (470 µE m-2 s-1), 250 µg/L, pH 7.5-8.6, 20 ° C) **≤ 1 hour** (mesocosm, sunlight (1275 - 3900 µE m-2 s-1), 25 µg/L)	Cardoza et al., 2005[1]
	C: 13.3 days (pond water, artificial UV-A light, 10 mg/L, pH 8.4); **47.4 days** (pond water, fluorescent light, 10 mg/L, pH 8.4); **<1 hour** (sterile pond water, sunlight, 10 mg/L)	Lin et al., 2010[1]
	C: ≈ 1 min (pure water, simulated sunlight (300 - 800 nm, 500 Wm-2), 100 µg/L, pH 4 and 8. 25 ° C) A few minutes (river water, simulated Sunlight (300 - 800 nm, 500 Wm-2), 100 µg/L, pH 8, 25 ° C)	Babić et al., 2013[1]
	C: 46.4 min (pH 5); **9.0 min** (pH 7); **23.1 min** (pH 9) (all exp, 5 mg/L)	Gagliano and McNamara, 1996 (Bayer Report Nr. 106563)[1]
	C: 1.5 hours (pure water, artificial sunlight (200 Wm-2), 10 mg/L)	Burhenne et al., 1997[1]
	C: 0.31 - 3.7 days (in surface waters calculated from Quantum Yield for different seasons)	Bayer AG, 1990a[1]
	C: > 2 months (river water and pure water, sunlight, 1 mg/L)	Turiel et al., 2005[1]
	C-HCl: 22.9 min (pure water, similar to sunlight (290-420 nm, 8.3 Wm-2), ≤ 1.3 mg/L, pH 6.44); **19.3 min** (fresh water, sim. Sunlight (290-420 nm, 8.3 Wm-2), ≤ 1.3 mg / L, pH 8.03); **26 min** (salt water, sim. Sunlight (290-420 nm, 8.3 Wm-2), ≤ 1.3 mg / L, pH 7.81)	Linke et al., 2010[1]
	C: 46 min >99% (DT_{99}) of parent compound was eliminated. (UV lamp, Millipore water (150 W) 20 mg/L)	Toolaram et al., 2016
Biodegradation	No biodegradation for 40 days (OECD 301D) No biodegradation for 40 days (OECD 301D) No biodegradation for 28 days	Kümmerer et al., 2000 Al-Ahmad et al., 1999 Girardi et al., 2011

1 = Data collected from Swiss Ecotox Centre (2013).

7. ENVIRONMENTAL CONCENTRATIONS

Ciprofloxacin has been measured in surface water, ground water, drinking water, sediment, soil, and in effluents, inlets, and sludge from wastewater facilities. The database "Pharmaceuticals in the environment" developed by UBA provide measured environmental concentrations (MEC) from Europe, Asian, North and South America, although it generally lack references to the original studies which in turn makes it difficult to scrutinize the underlying data. Swedish measurements and the highest measured concentrations for different matrixes from the database "Pharmaceuticals in the environment" are presented in table 3, with additional measured concentrations from the literature and from the Swedish Environmental Research Institute (IVL) screening database. The highest available environmental measurements are near pharmaceutical manufacture. Lemus et al. (2009) reported ciprofloxacin levels of 2.45-6.24 ng/L in Griffon vulture and Red kite eggs. However, these results should not be taken into account since several papers by this researcher has been retracted for suspected data fabrication, and including a fake author on his papers (Retraction Watch, n.d.). These data are therefore not included in table 3 below. Predicted environmental concentrations (PEC) found in the literature are presented in table 4. However, several of these predictions does not take into account the degradation within the body or elimination within the treatment plants. The most realistic predicted surface water concentrations are those reported by Johnson et al. (2015) which addressed these issues.

Table 3. Examples of measured environmental concentrations of ciprofloxacin.

Compartment	**Measured environmental concentration (MEC)**	**Reference**
Freshwater (surface) (ng/L)	66 (mean) 160 (max) (Sweden)	TemaNord, 2012[1]
	17.8 (mean) (Sweden, Umeå)	Khan et al., 2012[1]
	32 (median) 380[2] (max) <10 (min) (Sweden)	IVL screening database
	20-40.7 (Gościcina River, Poland)	Wagil et al., 2014
	5-18 (Glatt river, Swizerland)	Golet et al., 2002
	60 (Germany)	Kümmerer et al., 2000
	9 (Germany)	Christian et al., 2003
	37.5 (max) (river Italy Pisa)	Zuccato et al., 2010
	16 (max) (river Italy, Piacenza)	Zuccato et al., 2010
	26 (Italy)	Calamari et al., 2003
	80-119 (River Portugal, Downstream of STP)	Pena et al., 2007
	<0.41-119 (Brazil)	Locatelli et al., 2011
	30 (USA)	Sanderson et al., 2003
	2.9 (min), 43 (max), 9.5 (median) (lake, China)	Xie et al., 2017
	110 -130 (river, China)	Luo et al., 2011
	2.5E+06 – 6.5E+06 (lake, India near pharmaceutical manufacture)	Fick et al., 2009
	10.000- 2.5E+0.6 (river, India near pharmaceutical manufacture)	Fick et al., 2009
	5E+06 (river, India near pharmaceutical manufacture)	Gothwal and Shashidhar, 2017

Ground water (ng/L)	BDL (Sweden)	TemaNord, 2012[1]
	<10 and 25 (Near STP, Sweden, Uppsala)	IVL screening database
	64.5- 323.8 (mean) (Spain)	Cabeza et al., 2012[1]
Wells (ng/L)	44- 14 000[3] (India)	Fick et al., 2009
Marine waters (coastal and/or transitional) (ng/L)	66 (max) 31 (mean) (China)	Zhang et al., 2012[1]
	10-26 (mean) (China)	Na et al., 2011[1]
Wastewater treatment plant/sewage treatment plant effluent (ng/L)	54 (Sweden)	Wennmalm and Gunnarsson, 2009
	<15 (three STP in Sweden)	Bengtsson-Palme et al., 2016
	19 (median) 210 (max) <10 (min) (Sweden)	IVL screening database
	61 (Sweden, Umeå)	Lindberg et al., 2014
	7-18 (Sweden, Sthlm)	Lindberg et al., 2005
	13-32 (Sweden, Gbg)	Lindberg et al., 2005
	32-60 (Sweden, Umeå)	Lindberg et al., 2005
	7-14 (Sweden, Kalmar)	Lindberg et al., 2005
	7 (Sweden, Floda)	Lindberg et al., 2005
	43 (max) 34 (mean) (Sweden, Gbg)	Skoglund et al., 2008[1]
	10 (max) 8.5 (mean) (Sweden, Skövde)	Skoglund et al., 2008[1]
	31 (max) 21.6 (mean) (Sweden, Borås)	Skoglund et al., 2008[1]
	10 (max) (Sweden, Skövde)	Fick et al., 2011[1]
	82.7 (mean) (Sweden, Umeå)	Khan et al., 2012[1]
	43 (max) (Sweden, Ekebyhov)	Paxeus, 2010[1]
	53 (max) (Sweden, Djurö)	Paxeus, 2010[1]
	183 (max) (Sweden, Bromma)	Paxeus, 2010[1]
	163 (max) (Sweden, Henriksdal)	Paxeus, 2010[1]
	41 (max) 20 (mean) (Sweden)	TemaNord, 2012[1]
	60 (max) 23 (median) (Sweden)	Sadezky et al., 2008[1]
	130 (max) 60 (mean) (Finland)	Vieno et al., 2007
	14 (mean) (UK)	Singer et al., 2014
	52 (mean) (UK)	Singer et al., 2014
	137 (mean) ng/L (Portugal)	Pereira et al., 2015
	46- 499 (range) ng/L (Italy)	Al Aukidy et al., 2012
	148 (Italy, Varese)	Zuccato et al., 2010
	140 (max) 72 (mean) (EU, Brazil, North America)	Miège et al., 2009
	591 (max) 199 (mean) (Greece)	Papageorgiou et al., 2016
	260 (max) 67 (mean) (USA)	Kostich et al., 2014
	118 (mean) (Canada)	Miao et al., 2004
	32 000- 99 000 (max) (Hospital, Brazil)	Martins et al., 2008[1]
	28E+06 – 31E+06 (Pharmaceutical manufacture, India)	Larsson et al., 2007[1]
	14E+06 (India)	Fick et al., 2009[1]
Wastewater treatment plant/sewage treatment plant inlet (ng/g)	470 (max) 30 (min)	IVL screening database
	1100 and 2400 (hospital, Sweden)	TemaNord, 2012[1]
	194 (max) 133 (mean) (Sweden)	TemaNord, 2012[1]
	1900 (max) (Sweden, Henriksdal)	Paxeus, 2010[1]
	600 (max) (Sweden, Bromma)	Paxeus, 2010[1]
	1242 (max) (Sweden, Djurö)	Paxeus, 2010[1]
	740 (max) (Sweden, Ekebyhov)	Paxeus, 2010[1]
	248 (mean) (Sweden, Gbg)	Skoglund et al., 2008[1]
	270 (max) (Sweden, Skövde)	Fick et al., 2011[1]
	<15-910 (three STP in Sweden)	Bengtsson-Palme et al., 2016

	3700 (mean) (Sweden)	Zorita et al., 2009[1]
	155 000 (max) (hospital, Brazil)	Martins et al., 2008[1]
	17.5E+06 (industrial sewage, Croatia)	Dolar et al., 2012[1]
Wastewater treatment plant sludge (µg/kg)	4434-6188 (primary sludge, Sweden)	Bengtsson-Palme et al., 2016
	5123-12 197 (surplus sludge, Sweden)	Bengtsson-Palme et al., 2016
	7705-14 286 (digested sludge, Sweden)	Bengtsson-Palme et al., 2016
	8859 (kemikond-treated sludge, Sweden)	Bengtsson-Palme et al., 2016
	8800 (median) (Sweden, Henriksdal)	NORMAN, 2012[1]
	6300 (max) (Sweden, Bollebyggds)	NORMAN, 2012[1]
	3450 (median) (Sweden)	NORMAN, 2012[1]
	700 (max) (Sweden, Ellinge)	NORMAN, 2012[1]
	500 (max) (Sweden)	Lindberg et al., 2005[1]
	1200 (median) (10 Swedish STP)	Olofsson et al., 2012[1]
	7.4 (max) 3.6 (mean) (Sweden)	TemaNord, 2012[1]
	450 (max) (Sweden, Skövde)	Fick et al., 2011[1]
	4015-97 460 (Hospital, Norway)	Thomas et al., 2007
	10 800 (max) (China)	McClellan and Halden, 2010[1]
Sediment (µg/kg)	<20 ng/g organic matter (Sweden, Skövde)	Kristiansson et al., 2011
	130 (max) 44 (mean) dw (Norway)	TemaNord, 2012[1]
	1.3- 34.1 (Turkey, Istanbul)	Okey et al., 2012
	914 000 (downstream) 7100 (upstream) µg/kg organic matter (India)	Kristiansson et al., 2011
	22 (max) 1.9 dw (median) (China)	Xie et al., 2017
	12.1- 42.9 dw (max) (China)	Shi et al., 2014
	11-55 (China)	Luo et al., 2011[1]
	23.2 dw (China)	Goa et al., 2012
	0.88 dw (mean) (China)	Bai et al., 2014
Biota (µg/kg)	7-8.5 ww (max) (muscle of *Gadus morhua,* Sweden)	Hallgren and Wallberg, 2015
	12.5- 18.5 (*Oncorhynchus mykiss*, fish farm from Polish river)	Wagil et al., 2014
	4.2 (mean) 12.5 (max) dw (fish muscle, river in China)	Goa et al., 2012
	64 dw (median) (brain of Common carp, Lake in China)	XIe et al., 2017[4]
	130 dw (median) (liver of Silver carp, Lake in China)	
	77 dw (median) (liver of Crucian carp, Lake in China)	
	40 dw (median) (brain of Redfin culter, Lake in China)	
	96 dw (median) (liver of Yellow catfish, Lake China)	

1 = Data provided by the UBA database. 2 = Skövde WWTP upstream. Unrealistic high value (higher than measurements from effluents in Sweden). 3 = Wells used for human drinking water contained up to 1100 ng/L. 4 = The organ with highest concentrations reported in the table. dw= dry weight. ww= wet weight.

Table 4. Examples of predicted environmental concentrations of ciprofloxacin.

Compartment	**Predicted environmental concentration (PEC)**	**Master reference**
Freshwater (surface) (ng/L)	427	FASS, 2013
	0–40 (Predicted mean of European rivers)	Johnson et al., 2015
	630-670	Halling-Sørensen et al., 2000
	60	Kümmerer et al., 2000
	7500 (EMEA guideline, Norway)	Grung et al., 2008
	270 (Conventional method, Norway)	Grung et al., 2008
Wastewater treatment plant effluents (ng/L)	2720 (worst case) 1200 (refined)	Lindberg et al., 2006
	600	Kümmerer et al., 2000
	2000-30 000 (theoretical concentration in hospital wastewater)	Kümmerer et al., 2000
	195 (surface waters via STP, Korea)	Ji et al., 2016
Marine waters (coastal and/or transitional)	Not investigated	
Sediment	Not investigated	
Biota (freshwater)	Not investigated	
Biota (marine)	Not investigated	
Biota (marine predators)	Not investigated	

8. ECOTOXICITY OF CIPROFLOXACIN

The mode of action of ciprofloxacin involves inhibition of the bacterial enzymes DNA gyrase and topoismerase IV, which are enzymes required for replication and transcription in prokaryotic cells (Hooper et al., 1987; Fisher et al., 1988; Robinson et al., 2005). Quinolone antibiotics interact differently to the eukaryotic enzyme topoisomerase II, primarily because of differences of the DNA structure, therefore the potential of genotoxic effects in eucaryotes is considerably lower compared to prokaryotic organisms (Toolaram et al., 2016). The mode of action in eucaryotes is less clear however, in plants (macrophytes) ciprofloxacin has been suggested to interfere with photosynthetic pathways (Aristilde et al., 2010) possibly caused by oxidative stress (Gomes et al., 2017).

8.1 Ecotoxicity of heterotrophic bacteria

Ecotoxicity studies with autotrophic bacteria (i.e. cyanobacteria) can be used instead of studies with green algae for both acute and chronic QS derivation (European Communities, 2011). In addition, EC_{50} values for bacteria may be used in the derivation, but cannot substitute any of the other trophic levels (algae, Daphnia or fish). Studies with heterotrophic bacteria should be considered as short-term tests, and NOEC/EC_{10} values for bacteria should not be used in derivations when using assessment factors, but are relevant as inputs in a species sensitivity distribution (SSD). In terms of heterotrophic bacteria, the purpose is not to assess the risk for individual species rather the functionality of the microbial community (although, in case of antibiotics, a primary objective is to assess the risk of promoting resistance development, see section 9).

8.1.1 Single-species ecotoxicity (growth inhibition)

All single-species ecotoxicity studies using heterotrophic bacteria are presented in supportive information (table S1). The lowest bacteria results were reported by Załeska-Radziwiłł et al. (2014) in a growth inhibition test (ISO 107122) for *Pseudomonas fluorescens* with EC_{50} of 0.175 µg/L (NOEC 0.005 µg/L). This value was lower compared to effect value found in the Swiss EQS dossier, IC_{50} of 80 µg/L for *Psedomonas putida* (Al-Ahmad et al., 1999). However, Załeska-Radziwiłł et al. (2014) also reported that ethinylestradiol affected *Aliivibrio fisheri* at concentrations from 1.5 ng/L (NOEC), which is highly inconsistent with other literature and the fact that ethinylestradiol do not have a drug target in bacteria. Taking this into consideration, the study as a whole is questionable. Załeska-Radziwiłł et al. (2014), Yang et al. (2016) and Nałęcz-Jawecki et al. (2010) performed Microbial Assay for Risk Assessment (MARA) using 11 microbial strains, and reported lowest effect values for *Citrobacter freundii* (4.6- 46.4 µg/L) and *Delftia acidovorans* (6.2-36 µg/L). Yang et al. (2016) reported concentration interval and picture of the MARA assay, and their results were in the same interval although Załeska-Radziwiłł et al. (2014) reported somewhat lower EC_{50} values. The lowest effect value by Yang et al. (2016) was EC_{50} <29.8 µg/L for *D. acidovorans.*

8.1.2 Investigations of functionality of microbial communities, microcosms and mesocosms studies

Several studies has showed that ciprofloxacin can modify the microbial community structure (i.e. abundance and diversity) in water, sediment, and soil (Näslund et al., 2008; Códova-Kreylos and Scow, 2007; Gonzalez-Martinez et al., 2014; Maul et al., 2006; Weber et al., 2011; Cui et al., 2014; Girardi et al., 2011) from concentrations of 200 µg/L and 0.1 µg/kg (Näslund et al., 2008). In terms of broader microbial functionality affected by ciprofloxacin; nutrient regeneration, organic matter mineralization, and pollutant (pyrene) degradation has been investigated.

In synthetic wastewater ciprofloxacin reduced nitrification, denitrification, and phosphorus uptake at concentrations of 200-350 µg/L. This was accomplished by reduction of either ammonium oxidation bacteria, denitrifying bacteria or polyphosphate accumulating organism (Gonzalez-Marinez et al., 2014; Yi et al., 2017).

Girardi et al. (2011) found that the microbial activity, measured as acetate mineralization inhibition, was 75% lower (after 29 days) at ciprofloxacin concentration of 18 000 µg/L (single concentration used) compared to the control. In a 12 days microcosms, concentrations of 90 µg/L (nominal) significantly altered microbial (detrivorous) communities compared to the control and two tested concentrations (1.0 and 10 µg/L). In addition, the relative microbial respiration (measured with Ecoplates) for carbohydrate substances was significantly affected at 90 µg/L with 2.7 to 3.5 fold lower respiration compared to control and the lower concentrations. There was no significantly reduction of respiration in terms of carboxylic acids, amino acids, or polymer carbon substances (Maul et al., 2006). Weber et al. (2011) conducted wetland mesocosms (planted with *Phragmites australis* seeded with activated sludge from a WWTP) exposed to 2000 µg/L of ciprofloxacin. A temporary decrease in the activity and overall catabolic capabilities (based on reduced carbon source utilization) was observed of the bacterial communities as well as decreased overall diversity of bacterial operational taxonomic units. However, after 2-5 weeks of recovery the communities reverted to levels comparable to those unexposed of ciprofloxacin. Johansson et al. (2014) investigated the microbial carbon utilisation of marine biofilms (using Ecoplates). They found that carbon utilization was inhibited with 72h EC_{50} , EC_{10} and NOEC of 163, 15 and 9 µg/L, respectively, indicating that this is a more sensitive endpoint compared to e.g. nitrogen recycling.

Näslund et al. (2008) investigated the effects on pyrene degradation in marine sediment in a microcosm experiment with ciprofloxacin in the overlying water. The results showed a dose-dependent reduction of pyrene degradation with NOEC and EC_{50} calculated to 200 and 570 µg/L (nominal), respectively, after 11 weeks exposure. This corresponds to estimated sediment concentrations of 0.1 and 0.4 µg/kg dw, respectively.

8.2 Aquatic ecotoxicity to cyanobacteria, algae, macrophytes, invertebrates and vertebrates

Besides heterotrophic bacteria, cyanobacteria and macrophytes are also sensitive to ciprofloxacin with EC_{50} of 36.3 and 174 µg/L (table 5) and EC_{10} of 4.47 and 149 µg/L (table 6), respectively. A wetland mesocosms showed reduced growth, porosity, and evaporation for the mycrophyte *Phragmites australis* exposed to 2000 µg/L (single concentration) for 5 days (monitored for 100 days) (Weber et al., 2011). The effect values found for macroalgae under standard laboratory conditions suggest that they are less sensitive than cyanobacteria and macrophytes. In a microcosm, using algae collected at sites upstream and downstream from WWTP, ciprofloxacin did not negatively affect algal community growth or biomass (concentrations of 0.015-1.5 µg/L). However, shifts in the community structure at both sites were observed as well as a reduction in final algae genus diversity (Wilson et al., 2003).

For fish, no lethal effects were observed in neither acute nor chronic studies. Chronic traditional endpoints such as growth were significantly affected at 1000 µg/L, with increased length and weight of *Cyprinus carpio* (early-life stage) (Zivna et al., 2016). Zivna et al. (2016) also reported greater hatching rate at all concentrations (1-3000 µg/L), reduced development in some larvae stages at 1-500 µg/L, and accelerated development at 1000-3000 µg/L. Plhalova et al. (2014) did not observe effects on growth of *Denio rerio* (juveniles) at concentrations up to 3000 µg/L. Further, Zivna et al.

(2016) and Plhalova et al. (2014) investigated the activity of some oxidative stress markers and enzyme activity in fish. They received dispersed results, and the reported effects were not always dose-response related.

Invertebrates seem to not be among the sensitive taxa, with lowest NOEC of 1600 µg/L for *Daphnia magna* (Martins et al., 2012). In a sediment and water microcosms there was no effect seen on growth for either *Gammarus spp.* or *Lepidostoma liba* (macroinvertebrates) exposed to 0.9 and 90 µg/L, respectively, for 45 days (Maul et al., 2006). Taken together, there is no consistent and convincing data that ciprofloxacin will affect crustaceans or fish at low µg/L concentrations. However, in a study investigating effects of *Rhinella arenarum* (Amphibia) exposed to 1, 10, 100 and 1000 µg/L for 96 hours, ciprofloxacin showed reduced larvae length at 10 µg/L. A significant development inhibition greater than 10% was observed for concentrations of 100 and 1000 µg/L and additionally, GST levels increased at 1000 µg/L (Peltzer et al., 2017).

8.3 Sediment ecotoxicity to invertebrates

Ciprofloxacin has potential to sorb to sediment, i.e. the cut off value of Log K_{OC} ≥3 from European Communities (2011) is met. The log K_{ow} value however, does not reveal evidence on accumulation. It has been shown that ciprofloxacin sorbs to sludge, sediments, and clay (Cardozoa et al., 2005; Lindberg et al., 2005; Golet et al., 2002; Córdova-Kreylos and Scow 2007). Only one sediment toxicity study was found, investigating reproduction effects of *Lumbriculus variegatus* and *Chironomus riparius* during 28 days exposure. Both species were exposed to 0.25, 0.5, 1.0, 2.0 and 4.0 µg/kg, which did not cause any significant effects. However, the bioavailability of ciprofloxacin was unclear and the chemical analysis only detected traces of ciprofloxacin (too low to quantify), and the results were based on nominal concentrations. The authors argued that the low detection of ciprofloxacin might be due to degradation and photolysis, or covalent binding to the sediment (Nentwig, 2008). There were no available studies enabling QS derivation for the sediment compartment.

9. POTENTIAL TO SELECT FOR ANTIBIOTIC RESISTANCE

9.1 Mechanisms for fluoroquinolone resistance

The main mechanisms behind acquired quinolone resistance are mutation in *gyr*A and *par*C (i.e. the genes encoding the target proteins), efflux mediated resistance, or target protection inferred by *qnr* genes that are often horizontally transferrable (Ruiz, 2003; Boulund et al, 2017). From an environmental perspective, resistance mechanisms that are based on mutations in pre-existing DNA are often less concerning than horizontally transferrable genes. Selection pressures from antibiotics in the environment that favour mutation based resistance is primarily a concern if the pathogens themselves thrive in the external environment. Selection of horizontally transferrable resistance mechanisms, such as the case is for the *qnr* genes, have the ability to move between strains species through e.g. plasmids and conjugative transposons. Hence, selection for such mechanisms in the external environment can become a health problem even if there is no pathogen present in that particular environment. Depending on the "ecological connectivity" these bacteria may, sometimes through several steps, transfer such genes into pathogens at other locations (Bengtsson-Palme et al., 2018).

Environmental bacteria are believed to form a vast source of resistance genes that over time, under a selection pressure of antibiotics, are transferred and established in pathogens (Gaze et al, 2013; Finley et al., 2013). In fact, *qnr* genes form one of the better examples where environmental *Schewanella* species are likely the original host for some forms. The *qnr* genes encode pentapeptide proteins that are believed to mimic the DNA spiral and thereby blocks the binding of quinolones to the target. The *qnr* genes are grouped into six classes: *qnrA, qnrB, qnrC, qnrD*, *qnrS and qnrVC* with a growing list of gene variants (Boulund et al., 2017). As these genes already circulate in the human gut flora, Bengtsson-Palme and Larsson (2015) argued that it is more likely that transfer of such well-known genes to pathogens would occur in the human gut compared to in the external environment. In contrast, the authors pointed out that the highest concern in the environment is probably the selection of those resistance genes that we have not (yet) encountered in pathogens.

9.2 Investigations of potential to develop resistance to Ciprofloxacin

Gullberg et al. (2011) showed in pairwise competition experiments with *E.coli* strains that selection for resistant bacteria occurred down to 0.23 µg/L, which was 100 times below the MIC of the susceptible strain. A minimal selective concentration (MSC) of 0.1 µg/L was estimated based on extrapolation between data points. Likewise, Liu et al. (2011b) used competition tests of wild-type and resistant strains of *E. coli*, which resulted in selection for resistance at 3 µg/L, approximately 1/5 of the MIC concentration.

Tello et al. (2012) used species sensitivity distributions (SSDs) of MIC_{50} and NOEC values from the EUCAST database to predict whether measured environmental concentrations may select for resistance. They found that the potential of affected fractions of bacteria in river sediments, swine faces lagoons, liquid manure and farmed soil to select for resistance were greater compared to aquatic compartments. The predicted PNEC for ciprofloxacin that can be derived from Tello et al. (2012) is approximately 0.1 µg/L. In the study by Bengtsson-Palme and Larsson (2016) the lowest MIC in the EUCAST database (covered 29 taxonomic genera from 18 families) was <2 µg/L, which is the lowest reported concentration in the EUCAST testing scale. The predicted lowest observed MIC based on species coverage was therefore extrapolated and estimated to 1.2 µg/L, which corresponds to the

estimated upper boundary for the MSC. PNEC for resistance selection was calculated by rounding down the size-adjusted lowest MIC prediction to the nearest concentration on the EUCAST testing scale (0.64) and applying AF 10, resulting in a PNEC of 0.064 µg/L. The PNEC was recommended for implementations of emission limits. The lowest observed MIC value of 2 µg/L, was close to measured effluent concentrations in some studies, suggesting that environmental concentrations may pose a risk (Bengtsson-Palme and Larsson, 2016).

Karupner et al. (2018) investigated selective concentration for ciprofloxacin resistance in complex aquatic bacterial communities using biofilms in flow-through systems with ciprofloxacin concentrations of 0, 0.1, 1 and 10 µg/L. Endpoints investigated included taxonomic composition, within-species selection of resistance in *E. coli*, chromosomal resistance mutations and transferrable resistance genes. The results showed that the taxonomic composition significantly changed at 1 µg/L, which is just below the lowest MIC reported in the EUCAST database. At concentrations of 10 µg/L the resistant fraction and relative abundance of mutations of *E. coli* significantly increased. Further analysis revealed that mobile quinolone resistance genes were enriched followed by ciprofloxacin exposure of 1 µg/L or higher concentrations (NOEC of 0.1 µg/L). Ten mobile quinolone resistance genes/gene cluster were detected of which the *qnrB, qnrD* and *qnrS* significantly increased with increasing ciprofloxacin concentration (with *qnrD* being most sensitive and increased at 1 µg/L). This complex aquatic biofilm approach was also compared to a simplified approach using planktonic test tube system of which the results showed that the fraction of resistant *E.coli* or resistant heterotrophic bacteria significantly increased at 5 µg/L. (Kraupner et al., 2018).

Berglund et al. (2014) investigated the effects of antibiotic mixtures (included ciprofloxacin) in a water and sediment microcosms over 100 days, with no effect on antibiotic resistance genes or integron abundance at nominal concentrations of 740 µg/L The highest ciprofloxacin concentration was 20 µg/L in the mixture compared to concentration of 0.1 µg/L suggested to select for antibiotic resistance in Gullberg et al. (2011). The absence of resistance development could however be explained by very limited bacterial growth (Berglund et al., 2014). Ciprofloxacin was only added at the start of the experiment, with sediment concentration either increasing or remaining unchanged over time, thus another possible explanation is that the antibiotics probably became unavailable rapidly due to adsorption. Studies on resistance patters of *E. coli* in influents versus effluents from sewage treatment plants show unclear results and have limitation in the experimental design, but the overall pattern seems to suggest no or minor changes (e.g. Reinthaler et al., 2003).

10. QUALITY STANDARDS FOR ECOTOXICITY AND RESISTANCE

Two different approaches of QS derivation were considered:
1) Conventional QS_{pelag} values, excluding bacteria species with the exception of cyanobacteria, and basing QS on conventional species (cyanobacteria is considered to have the same status as algae according to European Communities, 2011).

2) QS_R value for risk of antibiotic resistance based both on experimental derivation of Minimal Selective Concentrations in *E. coli* (Gullberg et al, 2011), experimental LOEC/NOEC data for resistance selection in aquatic biofilms (Karupner et al., 2018) complemented by distribution of MIC data across bacterial species and strains as suggested by Bengtsson-Palme and Larsson, 2016) (section 10.2).

10.1 Conventional QS_{pelag} values

10.1.1 Acute freshwater toxicity MAC-QS_{fw}

The total dataset of acute freshwater ecotoxicity studies for ciprofloxacin included cyanobacteria, protozoa, algae, higher aquatic plants, crustacean, insects, annelida, amphibians, molluscs, and fish (table S2). Nine peer-reviewed studies (32 effect values) were found in addition to the Swiss EQS dossier (Swiss Ecotox Centre, 2013), with lower effect values for algae and crustacean. There was no available acute data for fish showing a significant effect. Studies which were not assessed as "reliable with or without restrictions" by Swiss Ecotox Centre (2013) were excluded from the dataset. The studies showing lowest effect values for the different taxonomic groups are presented in table 5. *Anabaena flos-aquae* with EC_{50} of 36.3 µg/L was assessed as "reliable" (e.g. analytically confirmed concentrations).

MAC-QS_{fw} was based on the lowest cyanobacteria value with EC_{50} of 36.3 µg/L for *A. flos-aquae* (Ebert et al., 2011). AF 10 was used since the mode of action of Ciprofloxacin to cyanobacteria is known and because the most sensitive taxonomic groups (in this case cyanobacteria since heterotrophic bacteria was not considered in this approach) was included (European Communities, 2011). MAC-QS_{fw} was set to 3.63 µg/L.

Table 5. The lowest acute freshwater toxicity values representing the different taxonomic groups.

Taxonomic group	Species	Endpoint and Duration		Effect value (µg/L)	Guideline/ Comments	Reference
Cyanobacteria	*Anabaena flos-aquae*	Growth rate	72h EC_{50}	36.3	GLP/ OECD 201 Reliability evaluation: 1	Ebert et al., 2011[1]
Algae	*Pseudokirchneriella subcapitata*	Growth inhibition	72h EC_{50}	5013	OECD 201	Van Doorslaer et al., 2015
Higher plants	*Lemna minor*	Frond increase	7d EC_{50}[2]	174	Reliability evaluation: 2	Robinson et al., 2005[1]
Crustacean	*Daphnia curvirostris*	Immobilization	48h EC_{50}	14 450	OECD 202	Dalla Bona et al., 2014
Fish	*Pimephales promelas*	Survival	7d NOEC[2]	≥9000	Reliability evaluation: 2	Robinson et al., 2005[1]
Amphibian	*Rhinella arenarum*	Survival	96h NOEC	>1000	-	Peltzer et al., 2017

1 = Data and reliability evaluation collected from Swiss Ecotox Centre (2013). 2 = Ciprofloxacin-HCl-H_2O used as test substance, a factor of 0,859 was used to convert to ciprofloxacin.

10.1.2 Acute marine toxicity (MAC-QS_{sw})

There were three marine algae ecotoxicity studies available, all assessed as "not reliable" by Swiss Ecotox Centre (2013) (table S3). MAC-QS_{sw} was derived using MAC-QS_{fw} and an additional AF 10 giving a MAC-QS_{sw} of 0.363 µg/L (European Communities, 2011).

We have, however, no reason to believe that marine cyanobacteria should be more sensitive than freshwater cyanobacteria. In fact, quinolones (including ciprofloxacin) has showed to be less effective in seawater due to binding with cations such as Mg^{2+} and Ca^{2+}, resulting in increased MICs (for pathogenic bacteria) by a factor of 2-8 compared to freshwater (Smith et al., 1989, 1990; Lie et al., 2000, in Hagenbuch and Pickney, 2012). Additionally, the main protection goal is not individual bacteria but should rather be the function of aquatic marine microorganism ecosystems. The study by Johansson et al (2014) suggest EC_{50} and EC_{10} of 163 and 15 ug/L for marine biofilms. Therefore, the additional assessment factor used to estimate the MAC-QS for the marine environment can be questioned.

10.1.3 Chronic freshwater toxicity (AA-QS_{fw})

The total dataset of chronic freshwater ecotoxicity studies included cyanobacteria, algae, higher plants, crustacean, and fish (table S4). Five peer-reviewed studies (with 22 effect values) were found in addition to the studies from the Swiss dossier (Swiss Ecotox Centre, 2013). Studies which were not assessed as "reliable with or without restrictions" by Swiss Ecotox Centre (2013) were excluded from the dataset. The studies showing lowest effect values for the different taxonomic groups are presented in table 6. *Anabaena flos-aquae* with EC_{10} of 4.47 µg/L was assessed as "reliable" (e.g. analytically confirmed concentrations and the validity criteria according to OECD 201 were met).

AA-QS was based on *A. flos-aquae* with EC_{10} of 4.47 µg/L (Ebert et al., 2011). AF 10 was applied since the dataset includes data from at least three species representing three trophic levels (European Communities, 2011). This scenario gives an AA-QS_{fw} of 0.447 µg/L.

Table 6. The lowest chronic freshwater toxicity studies representing the different taxonomic groups.

Taxonomic group	Species	Endpoint and Duration		Effect value (µg/L)	Guideline/ Comment	Reference
Cyanobacteria	*Anabaena flos-aquae*	Growth rate	72h EC_{10}	4.47[1]	GLP/OECD 201; Reliability: 1	Ebert et al., 2011[2]
Algae	*Chlorella vulgaris*	Growth rate (nr. of cells)	96h EC_{10}[3]	1800	Reliability: 2	Nie et al., 2008[2]
Higher plants	*Lemna gibba*	Biomass (Wet weight)	7d EC_{10}	149	ASTM; Reliability: 2	Brain et al., 2004[2]
Crustacean	*Daphnia magna*	Size of neonates of the 1st Brood	21d NOEC[3]	1600	OECD 202; Reliability: 2	Martins et al., 2012[2]
Fish	*Cyprinus carpio* (eggs)	Growth	33d NOEC	1000	OECD 210	Zivna et al., 2016
Amphibia	*Rhinella arenarum*	Length	96h EC_{10}[4]	10	-	Peltzer et al., 2017

1 = Unpublished data by Bayer AG (n.d.) (see supporting information table S4) suggest NOEC of 1.2 µg/L. Not included in the derivation since no information regarding the study was available. 2 = Data and reliability evaluation collected from Swiss Ecotox Centre (2013). 3 = Ciprofloxacin-HCl used as test substance, a factor of 0.9 was used to convert to ciprofloxacin. 4 = EC_{10} estimated.

10.1.4 Chronic marine toxicity (AA-QS_{sw})

There were no available chronic marine toxicity studies. AA-QS_{sw} was derived using AA-QS_{fw} and an additional AF of 10, giving an AA-QS_{sw} of 0.0447 µg/L (European Communities, 2011).

However, since there are no reason to believe that marine cyanobacteria should be more sensitive than freshwater cyanobacteria (see section 10.1.2), the additional assessment factor used to estimate the AA-QS for the marine environment can be questioned.

10.2 QS_R value for risk of antibiotic resistance

The study by Kraupner et al. (2018) directly investigated resistance selection of ciprofloxacin in complex aquatic biofilms using a variety of endpoint. Phenotypic, within species selection of *E.coli* in the biofilms was not found until an exposure concentration of 10 µg/L (no changes found at 1 µg/L or lower). However, for both selection of mobile quinolone resistance genes and taxonomic changes the LOEC was 1 µg/L, endpoints that under a highly controlled setup as the one used was interpreted as relevant for risk for resistance selection. The NOEC for these endpoints was 100 ng/L. This is largely in line with the pairwise strain competition experiment by Gullberg et al. (2011) who observed a selective advantage for a specific resistant mutant *E. coli* at 230 ng/L in culture media and estimating an MSC to 100 ng/L. Still, despite the vastly different exposure setups, both studies derive rather similar data on what concentrations are selective (or not) for resistance.

The studies above, empirically deriving selective concentrations for resistance, are also supported by a more theoretical approach based on growth inhibition data (Bengtsson-Palme and Larsson, 2016). Available data from the EUCAST database covers a large number of species (70 species) with lowest MIC value of <2 µg/L for *Enterobacter cloacae, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Neisseria gonorrhoeae, and Neisseria meningtidis* (in total 3443 observations of which 3093 were of *N. gonorrhoeae*). 2 µg/L correspond to the lowest concentration in EUCAST, the true MIC for these species are therefore unknown. The EUCAST database primarily covers clinically relevant bacteria, however, it is reasonable that there are equally or more sensitive bacteria present in the environment. In fact, some of the pathogens covered have the ability to spread in aquatic environments (e.g. acinetobacter, eschrichia, enterobacter).

Based on the assumption that concentrations that completely inhibits growth of some bacteria strains would also provide a selective advantage for resistant strains and that there may be equally sensitive species in the environment as those covered in the EUCAST database, the lowest MIC value of <2 µg/L was used to derive QS_R. The AF to be applied on the MIC should reflect (1) the number of bacteria species with MIC data available in EUCAST, as coverage of few species would indicate that there is a greater likelihood for considerably more sensitive bacteria species present in the environment, and (2) that MSC is lower than MIC.

In the EUCAST database, MIC values are only reported down to 2µg/L, and it is hence possible that some of the investigated strains were in fact more sensitive. For those antibiotics where some strains were sensitive to 2 µg/L (which includes ciprofloxacin), Bengtsson-Palme et al. (2016) predicted a lowest MIC by extrapolating the log2-distance below the peak MIC value and the lowest MIC value for that antibiotic across all species. This resulted in a predicted lowest MIC of 1.2 µg/L within those species covered in EUCAST. For ciprofloxacin there is a very large number of species covered in EUCAST (n=70) and over 300,000 isolates studied. Hence, it does not seem justified to apply an

assessment factor based on limited number of species, i.e. (1). An additional AF of 10 was, however, applied to take into account that MSC is expected to be lower than the MIC, i.e. (2). Without any rounding down of numbers to match the EUCAST testing scale (as done by Bengtsson-Palme and Larsson, 2016), this results in a PNEC for resistance of 0.12µg/L.

This is very similar to the predicted MSC of 0.1 µg/L based on competition experiments in *E. coli* (Gullberg et al., 2011) and the experimentally derived NOEC for resistance selection in biofilms (Kraupner et al., 2018). Hence, all three approaches would support a QS_R of 0.1 µg/L. Resistance development can be a "one-time event", i.e. consequences from the emergence of a new form of resistance can be major and widespread, even from an evolutionary event that takes place at one time in one place (Bengtsson-Palme and Larsson, 2015; Larsson, 2014b). Also, the generation time of bacteria is very short compared to higher level organisms. It is therefore reasonable to consider the value 0.1 ug/L as a "maximum allowed concentration" rather than an average concentration that should not be exceeded. This value is lower than both MAC-QS and AA-QS calculated to protect aquatic ecosystems.

10.2 Summary and proposal for surface water

Taking both aspects (conventional ecotoxicology and antibiotic resistance) into account, two different types of QS values for surface water were calculated (table 8):

1) Conventional QS_{pelag} values, excluding bacteria species with the exception of cyanobacteria, and basing QS on conventional species (according to European Communities, 2011). MAC-QS for the limnic and marine environments were set to 3.6 ug/L and 0.36 ug/L, respectively. AA-QS for the limnic and marine environments were set to 0.45 and 0.045 ug/L, respectively.

2) QS_R value for risk of antibiotic resistance based on experimental derivation of Minimal Selective Concentrations in *E. coli* (Gullberg et al., 2011) and empirically derived LOEC and NOEC values for resistance selection in aquatic biofilms (Kraupner et al., 2018), complementedby predictions of PNEC for resistance based on distribution of MIC data across bacterial species and strains as suggested by Bengtsson-Palme and Larsson, 2016) (section 10.2).

Taking current knowledge, the assumptions and calculations made above into account, 0.1 µg/L is proposed as sufficiently protective for the aquatic environment in both short and long term, limnic and marine environment, as well as against resistance development via the aquatic environment (indirect protection of human health). From a precautionary perspective, one can argue for an additional safety margin for resistance selection (Kraupner et al., 2018). The coherence of available studies so far, however, seems to indicate that 0.1 µg/L is reasonably protective.

It seems reasonable that protection of environmental side-effects in addition to resistance should be covered (Le Page et al., 2017; Bengtsson-Palme and Larsson, 2018). Studies investigating impacts on ecosystem functions (section 8.1.2) with lowest effect values of EC_{10} of 15 µg/L for the endpoint inhibition of carbon utilization (Johansson et al., 2014) is 100 fold higher than QS_R. Also, the QS_R is well below EC_{50} values from single-species bacteria studies (table S1) with conventional endpoint growth inhibition, although it is difficult to predict impacts on supportive ecosystem functions based on reduced growth.

Table 8. Proposals of MAC-QS and AA-QS for conventional QS-values and QS_R

MAC-QS (µg/L)		AA-QS (µg/L)		MAC-QS_R (µg/L) (based on MIC)
Freshwater	**Marine**	**Freshwater**	**Marine**	0.1
3.63	0.36	0.447	0.0447	

11. SECONDARY POISONING

Bioaccumulation field studies shows that ciprofloxacin has a BAF >100, i.e. potential to bioaccumulate, and does therefore fulfil the criteria for secondary poisoning according to European Communities (2011). The log K_{ow} or estimated BFC values however, does not reveal evidence on accumulation. Ciprofloxacin is classified as suspected to be toxic to reproduction according to the CLP and REACH legislation.

Studies on oral toxicity of ciprofloxacin includes effects on body weight, organ weight, behavioural parameters, neurological effects, histopathological changes, chondrotoxicity, and reproductive toxicity (table 12). Ilgin et al. (2015) concluded that ciprofloxacin in repeated doses (20 mg/kg/d) triggered depression, anxiety behaviours, and increased oxidative stress in female rats. Stahlmann et al. (2000) observed that dogs treated with 200 mg/kg/bw for 5 days had distinct pathological alterations (e.g. chondrocytes) compared to dogs treated with 30 mg/kg/bw. Keutz et al. (2004) showed that ciprofloxacin induced characteristic arthropathy in dogs treated with 30 mg/kg/bw. Ciprofloxacin in a subacute toxicity test as a positive control (15 mg/kg/d), caused decreased body weight in male mice (during day 6-10 and 12-16) (Khasawneh et al., 2015). Other observations include decreased organ weight (liver, kidneys, spleen, heart, and lungs) in female mice, and histopathological changes (liver, kidney, spleen, and lungs) in both sexes. However, since this single dose was administrated as a positive control it was not possible to determine a NOEL (Khasawneh et al., 2015).

Several reproduction toxicity studies are available with dispersed results. Investigations of cynomolgus monkeys administrated with 200 mg/kg/day from day 20 to 50 of pregnancy yielded no indications on embryo toxicity or teratogenicity. Endpoints investigated were physiological development of embryo or fetus, and increase in abortions. Further, no effect on progesterone levels were observed (Schluter, 1989). Likewise, there was no evidence of teratogenicity in mice treated with 25 mg/kg/day from gestation day 6-14 (Jahangir and Islam, 2006). Contrary, rats administrated with 15, 30 and 60 mg/kg/day from 6 to 12 days of gestation showed signs of embryo toxicity and teratogenicity at all doses. Endpoints investigated were; weight gain, incidence of abortions, litter size, and mean weight of pups (Siddiqui and Naqvi, 2010). Khaki et al. (2008) observed significant decrease of sperm concentrations, motility and viability, significantly decrease in number of spermatogenic cells, and lower weight of testis in male rats at 12.5 mg/kg/day for 60 days. The results were consistent with Abd-Allah et al. (2000) although they received higher effect values using shorter duration (table 12). However, ciprofloxacin was only single dose administrated, therefore it was not possible to determine a NOEL. Lemus et al. (2009) reported residues of ciprofloxacin and enrofloxacin in unhatched eggs of avian scavengers, this was suggested to cause fatal embryo chondral damage. However, this data has been withdrawn due to data manipulation (Retraction Watch, n.d.).

11.1. Derivation of $QS_{biota\ sec\ pois}$

The dose of 15 mg/kg/day (table 12) reported by Siddiqui and Naqvi (2010) resulted in 8.7- 17.42% effects for different endpoints and showed a dose-response with increased ciprofloxacin, e.g. mean weight of pulps yielded 14.4% reduction at 15 mg/kg/day and 21.6 and 27.7% reduction at 30 and 60 mg/kg/day, respectively. It is not stipulated in European Communities (2011) how to proceed with datasets of which the lowest effect value is a LOEL. Using the same approach as for EC_x values in the

range of 10-20% i.e. divide by 2 and tabulate as NOEC (European Communities, 2011), gives a NOEL of 7.5 mg/kg/day.

Using NOEL of 7.5 mg/kg/day, a conversion factor of 10 and AF of 90 (reproduction study) gives a $QS_{biota\ sec\ pois}$ of 833 µg/$kg_{ww\ food}$. Using the worst-case assumption of BAF (2008 L/kg) or BAF for fish muscle (508 L/kg) (Xie et al., 2017) the corresponding water concentrations was calculated to 0.42 and 1.64 µg/L, respectively. The calculated water concentrations are uncertain since these BAF are not representative of whole body BAF (see section 6.1) and therefore not suitable to use when converting to water concentrations. Therefore, QS for secondary poisoning was only proposed expressed as biota standard (wet weight in food). However, the worst-case calculation is in the same range as AA-QS_{fw} and above QS_R i.e. possible risk for secondary poisoning is believed to be covered by these derivations.

Table 12. Mammal toxicity studies for ciprofloxacin.

Species	Endpoint/Effects & Duration		Dose (mg/kg bw/d)	Reference
Mice (BALB/c)	Body weight, organ weight, histopathological changes	21d LOEL[1 2]	15	Khasawneh et al., 2015
Mice (newborn)	Weight gain, joints effects, liver development, cardiorespiratory and psychomotor development.	NOEL (LOEL)	30 (100)	Bourgeois et al., 2016
Wistar rats (Female)	Behaviour and neurological adverse effects	14d LOEL[1]	20	Ilgin et al., 2015
SD rats (Male 4-week old)	Cartilage alterations	7d LOEL (NOEL)[1]	800 (400)	Li et al., 2004
	Decreased thickness of articular cartilage of meoral condyle	7d LOEL (NOEL)[1]	800 (400)	
Rats	Decrease (and damage) in articular cartilage	15d LOEL[1] (single dose)	20	Halawa, 2010
Beagles dogs	Cleft formation and erosion of joint cartilage. Pathological alterations.	5d [3]	200	Stahlmann et al., 2000
Beagle dogs (juvenile)	Arthrotoxicity	14d LOEL (NOEL)[1]	30 (10)	Keutz et al., 2004
Rats	Biochemical parameters, hyaline degeneration and fibre disarrangement	21d LOEL[1] (single dose)	50	Olcay et al.,, 2011
Mice	Teratogenicity (gestation days 6-14)	21d LOEL	>25	Jahangir and Islam, 2006
Wistar rats (male)	Sperm concentration, motility and viability. No spermatogenic cells. Testis weight, epididymis and seminal vesicle	60d LOEL[1] (single dose)	12.5	Khaki et al., 2008
Wistar albino rats (male)	Total number of sperms, motility and daily sperm production	15 d LOEL[1] (single dose)	135	Abd-Allah et al., 2000
Wistar albino rats	Reproduction toxicity, Teratogenicity	LOEL (gestation days 6-12)	15	Siddiqui and Naqvi, 2010
Cynomolgus	Embryo toxicity and	LOEL	>200	Schluter, 1989

Species	Endpoint/Effects & Duration		Dose (mg/kg bw/d)	Reference
monkeys	teratogenicity			

1 = LOEL or NOEL not reported, significant results were used to predict values. 2 = Ciprofloxacin used as positive control. 3 = No information about significance.

12. IDENTIFICATION OF ISSUES RELATING TO UNCERTAINTY OF THE QS_R DERIVED

This QS derivation demonstrates that in the case of ciprofloxacin, the potential of developing resistance is the main driving factor (i.e. protection of human health). The derived QS_R is supported by independent investigations reporting PNECs, NOECs or related measures (MSC) in the same range (e.g. Gullberg et al., 2011; Tello et al., 2012; Bengtsson- Palme and Larsson 2016; Kraupner et al., 2018). The role of the selection pressure in the environment has repeatedly been recognized, although, there is a lack of knowledge of how and under which circumstances the environment contribute to the development of resistance (Bengtsson-Palme et al., 2018).

Within wastewater facilities, human pathogens and a wide diversity of environmental bacteria are present in high numbers, providing ample opportunities for transfer of resistance factors between bacteria (Lood et al., 2017; Rizzo et al., 2013). In downstream surface waters, human pathogens are usually considerably less common, and the levels of antibiotics lower, both due to removal in the treatment plants and due to dilution in the recipient. Consequently, the levels of ciprofloxacin within the wastewater treatment facilities may in fact be of higher importance for the risks of selecting for resistance, than the levels in surface water. In contrast to most other chemicals, the target organisms in focus for protection is actually present within the treatment plants. Therefore, even if removal is good at the wastewater treatment plants leading to environmental levels below those that are selective, resistance may still develop inside the treatment plans, and then spread to the external environment (Gao et al., 2012). A limited resident time and growth opportunities for many bacteria in wastewater treatment plants, however, would against such a risk. Correspondingly, even though concentrations of antibiotics in the environment are considerably lower than those found within treatment plants, the continuous release and persistent properties of ciprofloxacin may entail prolonged exposure covering many more generations of bacteria in the recipient.

There are also uncertainties due to the limited knowledge of ciprofloxacin’s bioavailability in sediments and impact on the functionality of microbial sediment communities.

13. IDENTIFICATION OF ANY POTENTIAL IMPLEMENTATION ISSUES IN RELATION TO THE QS_R DERIVED

Selection of resistance genes in the environment is currently not incorporated in any regulation or associated risk assessment frameworks. Action plans related to human health and antimicrobial resistance have been raised within the EU as well as on a global level with actions addressing the role of the environment (European Commission, 2017; WHO, 2015). Environmental priorities taken into account includes development of harmonised monitoring of antimicrobials and microorganisms resistant to antimicrobials in the environment, and to further explore and develop methodologies to evaluate risks to human and animal health (European Commission, 2017).

QS derivation is performed under the Water Framework Directive (2000/60/EC) using the European Communities' (2011) guidance document "Technical Guidance for Deriving Environmental Quality" which do not include the aspect of resistance development. In the light of new research, an approach that includes this aspect is proposed in this report. Antimicrobial resistance does not constitute *direct* risks for aquatic ecosystems or human health, however, it poses an *indirect* risk for human health (similarly to the assumption of secondary poisoning for human consumption of fishery products) through effects in the environment. Emergence of antimicrobial resistance is a complex task, reducing the emissions of antibiotics (and increased monitoring) needs to be combined with reducing the spread of antibiotic resistant bacteria to the environment and developing threshold guidance regarding the presence of these. Such regulations could include effluents, water recipients, and soil applications. Nevertheless, effective measures to control antibiotic usage in humans, pets and livestock are crucial, since this is the major drivers for modern emergence of resistance (Finley et al., 2013).

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15. SUPPORTIVE INFORMATION

This section summarizes all ecotoxicity data collected from Swiss Ecotox Centre (2013), additional data found in the literature, and MIC values collected from EUCAST database. Ecotoxicity studies of heterotrophic bacteria are presented in table S1, acute freshwater ecotoxicity data in table S2, acute marine data in table S3, chronic freshwater data in table S4 and MIC values in table S5.

Table S1. Ecotoxicity data for freshwater, marine, and sludge bacteria for ciprofloxacin.

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/ Comments	Reference
Freshwater bacteria					
Brevundimonas diminuta	Growth inhibition	EC_{50} (MTC)	1805.9 (374.4)	MARA test	Yang et al., 2016
Brevundimonas diminuta	Growth inhibition	18h EC_{50}-t	5057	MARA test	Załeska-Radziwiłł et al., 2014
Brevundimonas diminuta	Growth inhibition	18h NOEC	156.3	MARA test	Załeska-Radziwiłł et al., 2014
Citrobacter freundii	Growth inhibition	EC_{50} (MTC)	46.4 (39.8)	MARA test	Yang et al., 2016
Citrobacter freundii	Growth inhibition	18h EC_{50}-t	4.6	MARA test	Załeska-Radziwiłł et al., 2014
Citrobacter freundii	Growth inhibition	MTC	12	MARA test	Nałęcz-Jawecki et al., 2010
Citrobacter freundii	Growth inhibition	18h NOEC	0.04	MARA test	Załeska-Radziwiłł et al., 2014
Comamonas testosteroni	Growth inhibition	EC_{50} (MTC)	132.5 (82.8)	MARA test	Yang et al., 2016
Comamonas testosteroni	Growth inhibition	18h EC_{50}-t	56.1	MARA test	Załeska-Radziwiłł et al., 2014
Comamonas testosteroni	Growth inhibition	18h NOEC	2.4	MARA test	Załeska-Radziwiłł et al., 2014
Delftia acidovorans	Growth inhibition	EC_{50}/MTC	<29.8	MARA test	Yang et al., 2016
Delftia acidovorans	Growth inhibition	18h EC_{50}-t	6.2	MARA test	Załeska-Radziwiłł et al., 2014
Delftia acidovorans	Growth inhibition	MTC	36	MARA test	Nałęcz-Jawecki et al., 2010
Delftia acidovorans	Growth inhibition	18h NOEC	1.2	MARA test	Załeska-Radziwiłł et al., 2014
Enterococcus casseliflavus	Growth inhibition	EC_{50}/MTC	>67 595	MARA test	Yang et al., 2016
Enterococcus casseliflavus	Growth inhibition	18h EC_{50}-t	5023.6	MARA test	Załeska-Radziwiłł et al., 2014
Enterococcus casseliflavus	Growth inhibition	18h NOEC	156.3	MARA test	Załeska-Radziwiłł et al., 2014
Kurthia gibsonii	Growth inhibition	EC_{50} (MTC)	682.6 (430.8)	MARA test	Yang et al., 2016
Kurthia gibsonii	Growth inhibition	18h EC_{50}-t	370.4	MARA test	Załeska-Radziwiłł et al., 2014
Kurthia gibsonii	Growth inhibition	18h NOEC	4.9	MARA test	Załeska-Radziwiłł et al., 2014
Microbacterium sp.	Growth inhibition	EC_{50} (MTC)	301.5 (208.8)	MARA test	Yang et al., 2016
Microbacterium sp.	Growth inhibition	18h EC_{50}-t	144.1	MARA test	Załeska-Radziwiłł et al., 2014
Microbacterium sp.	Growth inhibition	18h NOEC	19.5	MARA test	Załeska-Radziwiłł et al., 2014
Pseudomonas aurantiaca	Growth inhibition	18h EC_{50}-t	150	MARA test	Załeska-Radziwiłł et al., 2014
Pseudomonas aurantiaca	Growth inhibition	18h NOEC	39.1	MARA test	Załeska-Radziwiłł et al., 2014
Pseudsomonas chlororaphis	Growth inhibition	EC_{50} (MTC)	149.1 (129.2)	MARA test	Yang et al., 2016
Pseudomonas fluorescens	Growth inhibition (optical density)	16h EC_{50}-t	0.18	ISO 107122-1994	Załeska-Radziwiłł et al., 2014
Pseudomonas fluorescens	Growth inhibition	16h NOEC	0.005	ISO 107122-1994	Załeska-Radziwiłł et al., 2014
Pseudomonas	Growth inhibition	16h IC_{50}	80	ISO 17012	Al-Ahmad *et al.,* 1999[1]

putida	(protein content)			(without light); Reliability evaluation: 2	
Pseudomonas putida	Growth	EC_{50}	9.3	Reliability evaluation: 4	Bayer AG, 1994[1]
Pseudomonas putida	Growth inhibition	EC_{50}	80	ISO 17 012	Kümmerer et al., 2000
Pseudomonas putida	Growth inhibition	EC_0	10		Kümmerer et al., 2000
Pseudomonas putida	Growth inhibition	EC_{50}	225		Girardi et al., 2011
Serratia rubra	Growth inhibition	EC_{50} (MTC)	255.1 (162.4)	MARA test	Yang et al., 2016
Serratia rubidaea	Growth inhibition	18h EC_{50}-t	265.6	MARA test	Załeska-Radziwiłł et al., 2014
Serratia rubidaea	Growth inhibition	18h NOEC	39.1	MARA test	Załeska-Radziwiłł et al., 2014
Staphylococcus warneri	Growth inhibition	EC_{50} (MTC)	407.6 (228.6)	MARA test	Yang et al., 2016
Staphylococcus warneri	Growth inhibition	18h EC_{50}-t	1528.6	MARA test	Załeska-Radziwiłł et al., 2014
Staphylococcus warneri	Growth inhibition	18h NOEC	9.8	MARA test	Załeska-Radziwiłł et al., 2014
Marine bacteria					
Aliivibrio fischeri	Growth	EC_{50}	6.7	Reliability evaluation: 4	Bayer AG, 1994[1]
Aliivibrio fischeri	Activated sludge respirometry test	EC_{50}	325 800	EPA 712-C-014 OCSPP 850.3300	Ortiz de García et al., 2014
Aliivibrio fischeri	Luminescence	NOEC	≥100 000	Reliability evaluation: 4	Zhang et al., 2012[1]
Aliivibrio fischeri	Luminescence	15min IC_{20}	93 000		Li et al., 2014
Aliivibrio fischeri	Luminescence	15min EC_{50}	211 800	ISO 11348-3:2007	Ortiz de García et al., 2016
Aliivibrio fischeri	Luminesces	15min EC_{50}	204 000	ISO 11348-3:2007	Ortiz de García et al., 2014
Aliivibrio fischeri	Luminescence	30 min EC_{50}[2]	10 400	Reliability evaluation: 2	Martins et al., 2012[1]
Aliivibrio fischeri	Luminescence	30min EC_{50}	>5900	ISO 11348-2; Reliability evaluation: 3	Hernando et al., 2007[1]
Aliivibrio fischeri	Luminescence	30min EC_{28}	5900	ISO 11348-2; Reliability evaluation: 3	Hernando et al., 2007[1]
Aliivibrio fischeri	Luminescence	30min EC_{50}	300	DIN 38412-L34 protocol	Wagil et al., 2014
Aliivibrio fischeri	Luminescence	15/30min EC_{50}-t	>100 000	LUMIStox	Załeska-Radziwiłł et al., 2014
Aliivibrio fischeri	Luminescence	30min LOEC (NOEC)	100 (10)	Microtox	Mater et al., 2014
Aliivibrio fischeri	Luminescence	24h EC_{50}-t	0.0137	LUMIStox	Załeska-Radziwiłł et al., 2014
Aliivibrio fischeri	Growth (optical density)	24h EC_{50}-t	1.4	LUMIStox	Załeska-Radziwiłł et al., 2014
Aliivibrio fischeri	Bioluminescence	24h NOEC	0.0015	LUMIStox	Załeska-Radziwiłł et al., 2014
Aliivibrio fischeri	Growth (optical density)	24h NOEC	0.0015	LUMIStox	Załeska-Radziwiłł et al., 2014
Sludge bacteria					
Activated sludge micro-organisms	Enzymatic (dehydrogenase activity)	30min/24h EC_{50}-t	>100 000	PN-C-04616-8:2008; 24.85% inhabitation	Załeska-Radziwiłł et al., 2014
Activated sludge micro-organisms	Enzymatic (hydrolytic activity)	30min/24h EC_{50}-t	>100 000	PN-C-04616-8:2008	Załeska-Radziwiłł et al., 2014
Sludge bacteria (water solution)	Growth inhibition	120h EC_{50}	6-10	without light	Halling-Sørensen et al., 2003

Sludge bacteria (water solution)	Growth inhibition	120h EC_{50}	7-8	with light	Halling-Sørensen et al., 2003
Sludge bacteria (activated sludge)	Growth inhibition	120h EC_{50}	8-25		Halling-Sørensen et al., 2003
Sludge bacteria	Growth inhibition	EC_{50}	610		Halling-Sørensen et al., 2000
Sludge bacteria	Growth inhibition	EC_{50}	64		Lykkeberg et al., 2007

1 = Data and reliability evaluations from Swiss Ecotox Centre (2013). 2 = Ciprofloxacin-HCl used as test substance, factor of 0.9 was used to convert to ciprofloxacin. MTC= microbial toxic concentration.

Table S2. Acute freshwater toxicity studies for ciprofloxacin.

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/ Comments	Reliability evaluation	Reference
Cyanobacteria						
Anabaena flos-aquae	Growth rate	72h EC_{50}	36.3	GLP/OECD 201; Based on measured concentrations	1	Ebert et al., 2011
Anabaena flos-aquae	Biomass	72h EC_{50}	10.3	GLP/OECD 201. The value for the growth rate is preferred (European Communities,2011)	1	Ebert et al., 2011[1]
Microcystis aeruginosa	Growth rate	72h EC_{50}	5[2]	OECD 201. Based on nominal concentrations	3	Halling-Sørensen et al., 2000[1]
Microcystis aeruginosa	Growth rate (fluorescence)	120h EC_{50}	15[3]	Effect value based on nominal concentrations (not stable)	3	Robinson et al., 2005[1]
Protozoa						
Blepharisma japonicum	Growth (Number of cells)	96h NOEC	≥0.9[2]	Based on nominal concentrations	3	Nentwig, 2006[1]
Tetrahymena thermophila	Growth	24h EC_{50}	>100 000	Based on nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Tetrahymena thermophila	Growth	24h NOEC	195	Based on nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Algae						
Chlamydomonas mexicana	Growth rate	96h EC_{50}	65 000			Xiong et al., 2017
Chlamydomonas mexicana	Growth rate	48h EC_{50}	55 000			Xiong et al., 2017
Chlorella vulgaris	Growth rate	96h EC_{50}	18 500[2]	Nominal concentration within 20%	2	Nie et al., 2008[1]
Chlorella vulgaris	Chlorophyll	96h EC_{50}	28 130[2]	Nominal concentration within 20%	2	Nie et al., 2008[1]
Chlorella vulgaris	Growth rate	72h EC_{50}	25 100[2]	OECD 201		Geiger et al., 2016
Chlorella vulgaris	Growth rate	96h EC_{50}	26 200[2]	OCED 201		Geiger et al., 2016
Desmodesmus subspicatus	Growth rate	72h EC_{50}	>8042	GLP/OECD 201; Based on measured concentrations	1	Ebert et al., 2011[1]
Desmodesmus subspicatus	Biomass	72h EC_{50}	>8042	GLP/OECD 201; Based on measured concentrations	1	Ebert et al., 2011[1]
Desmodesmus subspicatus	Growth rate	72h EC_{50}	>900 000[2]	DIN 38412	4	Bayer AG 1990b[1]
Desmodesmus subspicatus	Growth inhibition	72h EC_{50}	8800	OECD 201		Zhu et al., 2016
Pseudokirchneriella subcapitata	Growth rate	96h EC_{50}	3500[2]	OECD 201; Based on nominal concentrations	3	Martins et al., 2012[1]
Pseudokirchneriella subcapitata	Growth rate (fluorescence)	72h EC_{50}	16 100[2]	Effect value based on nominal concentrations (substance not stable)	3	Robinson et al., 2005[1]
Pseudokirchneriella subcapitata	Biomass	72h EC_{50}	6700	OECD 201; Based on nominal concentrations	3	Yang et al., 2008[1]
Pseudokirchneriella subcapitata	Growth	72h EC_{50}	2670[2]	OECD 201; Based on nominal concentrations	3	Halling-Sørensen et al., 2000[1]
Pseudokichneriella subcapitata	Growth inhibition	72h EC_{50}	11 300	EPS 1/RM/25		Magdaleno et al., 2015
Pseudokirchneriella subcapitata	Growth inhibition	72h EC_{50}	5013[2]	OECD 201		Van Doorslaer et al., 2015

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/ Comments	Reliability evaluation	Reference
Scenedesmus obliquus	Biomass	96h EC_{50}	126 320		3	Zhang et al., 2012[1]
Scenedesmus vacuolatus	Growth inhibition	24h EC_{50}	>1000	ISO Guideline 8692		Wagil et al., 2014
Higher plants						
Lemna gibba	Frond increase	7d EC_{50}	413	GLP/OECD 221; Based on measured concentrations	1	Ebert et al., 2011[1]
Lemna gibba	Frond increase	7d EC_{50}	62.5	GLP/OECD 221; based on measured concentrations	1	Ebert et al., 2011[1]
Lemna gibba	Frond increase	7d EC_{50}	697	ASTM E 1415-91; nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Biomass (Dry weight)	7d EC_{50}	499	GLP/OECD 221; measured concentrations	1	Ebert et al., 2011[1]
Lemna gibba	Biomass (Wet weight)	7d EC_{50}	698	ASTM E 1415-91; nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Chlorophyll (a) content	7d EC_{50}	1279	ASTM E 1415-91; nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Chlorophyll (b) content	7d EC_{50}	992	ASTM E 1415-91; nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Carotenoid content	7d EC_{50}	1762	ASTM E 1415-91; nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Frond increase (rate)	6d EC_{50}	219[2]	Nominal concentrations	3	Kolasińska et al., 2010[1]
Lemna gibba	Frond increase (rate)	6d EC_{50}	51[2]	Nominal concentrations	3	Kolasińska et al., 2010[1]
Lemna minor	Frond increase	7d EC_{50}	174[2]	Concentrations within 20%	2	Robinson et al., 2005[1]
Lemna minor	Frond increase	7d EC_{50}	170[2]	OECD 221; nominal concentrations	3	Martins et al., 2012[1]
Myriophyllum spicatum	Sprout length	14d EC_{50}	>63 530	GLP/ASTM E 1913-04; measured concentrations	1	Ebert et al., 2011[1]
Lemna minor	Growth inhibition	7d EC_{50}	340			Wagil et al., 2014
Annelida						
Lumbriculus variegatus	Survival	96h LC50	≥4800	Nominal concentrations	3	Nentwig, 2008
Crustacean						
Daphnia magna (< 24 h)	Immobilization	48h EC_{50}	58 800[3]	OECD 202; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna	Immobilization	48h EC_{50}	>9900	Measured concentrations	1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106596)[1]
Daphnia magna (< 24 h)	Immobilization	48h EC_{50}	>100 000	No light	3	Załeska-Radziwiłł et al., 2011
Daphnia magna	Immobilization	48h NOEC	≥9900	Measured concentrations	1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106596)[1]

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/ Comments	Reliability evaluation	Reference
Daphnia magna (< 24 h)	Immobilization	48h NOEC	≥9000[3]	Concentrations within 20%	2	Robinson et al., 2005[1]
Daphnia magna (< 24 h)	Immobilization	48h NOEC	≥50 000[2]	OECD 202; Nominal concentrations	3	Halling-Sørensen et al., 2000[1]
Daphnia magna (24 – 48 h)	Immobilization	24h EC_{50}	>12 000	ISO 6341; nominal concentrations	2	Dave und Herger, 2012[1]
Daphnia magna	Enzyme activity (galactosidase)	1h EC_{50}	3770	Fluorescence test	3	Załeska-Radziwiłł et al., 2011[1]
Daphnia magna (<24h)	Immobilization	48h EC_{50}	>1000	OECD 202		Wagil et al., 2014
Daphnia magna	Immobilization	48h EC_{50}	87 140	OECD 202		Dalla Bona et al., 2014
Daphnia curvirostris	Immobilization	48h EC_{50}	14 450	OECD 202		Dalla Bona et al., 2014
Hyalella azteca	Survival	96h LC_{50}	>10 200		1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106783)[1]
Hyalella azteca	Survival	96h NOEC	2240		1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106783)[1]
Insecta						
Chironomus riparius (Larvae)	Survival	24h LC_{50}	≥4800[2]		3	Nentwig, 2008[1]
Amphibians						
Xenopus laevis (Larvae)	Survival	96h NOEC	≥100 000	Nominal concentrations	3	Richards and Cole, 2006[1]
Xenopus laevis (Larvae)	Development	96h NOEC	≥100 000	Nominal concentrations	3	Richards and Cole, 2006[1]
Rhinella arenarum (larva)	Survival	96h NOEC	>1000	Concentrations within 20% of nominal	2	Peltzer et al., 2017
Fish						
Danio rerio	Survival	96h LC_{50}	1 000 000		4	Bayer AG, 1990c[1]
Danio rerio	Survival	96h LC_{50}	>100 000	Nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Danio rerio	Survival	96h NOEC	316 000		4	Bayer AG, 1990c[1]
Danio rerio	Survival	96h NOEC	≥90 000[2]	OECD 203; Nominal concentrations	3	Halling-Sørensen et al., 2000[1]
Gambusia holbrooki	Survival	96h LC_{50}	>54 000[2]	OECD 203; Nominal concentrations	2	Martins et al., 2012[1]
Lebistes reticulatus	Survival	96h LC_{50}	>100 000	Nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Lepomis macrochirus	Survival	96h LC_{50}	>9850	Measured concentrations	1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106791)[1]
Lepomis macrochirus	Survival	96h NOEC	≥9850	Measured concentrations	1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106791)[1]
Oncorhynchus mykiss	Survival	96h LC_{50}	9400	Measured concentrations	1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106775)[1]

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/ Comments	Reliability evaluation	Reference
Oncorhynchus mykiss	Survival	96h NOEC	≥ 9400	Measured concentrations	1	Gagliano and McNamara, 1996 (Bayer Report Nr. 106775)[1]
Pimephales promelas	Survival	7d NOEC	≥ 9000[3]	Concentrations within 20%	2	Robinson et al., 2005[1]
Pimephales promelas	Growth (weight)	7d NOEC	< 9000[3]	Increased weight; Concentrations within 20%	2	Robinson et al., 2005[1]

1 = Data and reliability evaluations from Swiss Ecotox Centre (2013). 2 = Ciprofloxacin-HCl used as test substance, factor of 0.9 was used to convert to ciprofloxacin. 3 = Ciprofloxacin-HCl-H_2O used as test substance, factor of 0.859 was used to convert to ciprofloxacin.

Table S3. Acute marine toxicity studies for ciprofloxacin.

Species	Endpoint & Duration		Effect value (µg/L)	Comments	Reliability evaluation	Reference
Algae						
Cylindrotheca closterium	Growth rate (no of cells)	96h EC_{50}	55 430	Nominal concentrations (risk for photo degradation)	3	Hagenbuch and Pickney, 2012[1]
Navicula ramosissima	Growth rate (no of cells)	96h EC_{50}	72 120	Nominal concentrations (risk for photo degradation)	3	Hagenbuch and Pickney, 2012[1]
Artemia salina	Immobilization	96h EC_{50}	>100 000	Nominal concentrations (risk for photo degradation)	3	Załeska-Radziwiłł et al., 2011[1]

1 = Data and reliability evaluations from Swiss Ecotox Centre (2013).

Table S4. Chronic freshwater toxicity studies for ciprofloxacin.

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/Comments	Reliability evaluation	Reference
Cyanobacteria						
Anabaena flos-aquae	Growth inhibition	NOEC	1.2	OECD 201; secondary literature	4	Bayer AG, n.d. (In Fass, 2013)
Anabaena flos-aquae	Growth rate	72h EC10	4.47	GLP/OECD 201; Measured concentrations	1	Ebert et al., 2011[1]
Anabaena flos-aquae	Biomass	72h EC10	5.65	GLP/OECD 201; Measued concentrations	1	Ebert et al., 2011[1]
Microcystis aeruginosa	Growth rate	14d NOEC	69.1	Not stable during test	3	Gagliano and McNamara, 1996 (Bayer Report Nr. 106627)[1]
Algae						
Chlorella vulgaris	Growth rate (no. of cells)	96h EC_{10}	1800[2]	Concentrations within 20%	2	Nie et al., 2008[1]
Desmodesmus subspicatus	Growth rate	72h NOEC	≥8042	GLP/OECD 201; Measured concentrations	1	Ebert et al., 2011[1]
Desmodesmus subspicatus	Biomass	72h NOEC	≥8042	GLP/OECD 201; Measured concentrations	1	Ebert et al., 2011[1]
Desmodesmus subspicatus	Growth rate	72h EC_{10}	27 000[2]	DIN 38412	4	Bayer AG 1990b[1]
Pseudokirchneriella subcapitata	Growth rate (no. of cells)	96h NOEC	900[2]	Nominal concentrations	3	Liu et al., 2011a[1]
Pseudokirchneriella subcapitata	Growth rate (no. of cells)	96h NOEC	981[2]	OECD 201; Nominal concentrations	3	Martins et al., 2012[1]
Pseudokirchneriella subcapitata	Photosynthesis (O2 production)	96h NOEC	450[2]	Nominal concentrations	3	Liu et al., 2011a[1]
Pseudokirchneriella subcapitata	Chlorophyll(*a*) content	96h NOEC	1350[2]	Nominal concentrations	3	Liu et al., 2011a[1]
Pseudokirchneriella subcapitata	Chlorophyll(*b*) content	96h NOEC	1350[2]	Nominal concentrations	3	Liu et al., 2011a[1]
Pseudokirchneriella subcapitata	Carotenoid content	96h NOEC	900[1]	Nominal concentrations	3	Liu et al., 2011a[1]
Pseudokirchneriella subcapitata	Biomass	72h NOEC	<5000	OECD 201; Nominal concentrations	3	Yang et al., 2008[1]

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/Comments	Reliability evaluation	Reference
Pseudokirchneriella subcapitata	Growth	14d NOEC	<12 800	Nominal concentrations	3	Gagliano and McNamara, 1996 (Bayer Report Nr. 106633)[1]
Pseudokirchneriella subcapitata	Growth inhibition	72h EC_{10}	3300	EPS 1/RM/25		Magdaleno et al., 2015
Higher aquatic plants						
Lemna gibba	Frond increase	7d EC_{10}	106	ASTM E 1415-91; Nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Biomass (Wet weight)	7d EC_{10}	149	ASTM E 1415-91; Nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Chlorophyll (*a*) content	7d EC_{10}	357	ASTM E 1415-91; Nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Chlorophyll (*b*) content	7d EC_{10}	247	ASTM E 1415-91; Nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Carotenoid content	7d EC_{10}	484	ASTM E 1415-91; Nominal concentrations	2	Brain et al., 2004[1]
Lemna gibba	Frond growth rate	7d NOEC	>10; <100	GLP/OECD 211; Measured concentrations	1	Ebert et al., 2011[1]
Lemna gibba	Frond increase	7d NOEC	>10; <100	GLP/OECD 211; Measured concentrations	1	Ebert et al., 2011[1]
Lemna gibba	Biomass (dry weight)	7d NOEC	>10; <100	GLP/OECD 211; Meausred concentrations	1	Ebert et al., 2011[1]
Lemna gibba	Frond growth rate	6d EC_{10}	42[2]	Nominal concentrations	3	Kolasińska et al., 2010[1]
Lemna gibba	Frond growth rate	6d EC_{10}	16[2]	Nominal concentrations	3	Kolasińska et al., 2010[1]
Lemna minor	Frond growth	7d NOEC	<45[2]	OECD 221; Nominal concentrations	3	Martins et al., 2012[1]
Myriophyllum spicatum	Sprout length	14d NOEC	980	GLP/ASTM E 1913-04; Measured concentrations	1	Ebert et al., 2011[1]
Crustacean						
Daphnia magna	Reproduction	28d EC_{50}	14 000	Number of offspring / individual; Nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Daphnia magna	Reproduction	28d NOEC	156	Number of offspring / individual; Nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Daphnia magna	Size of neonates of the 1st Brood	21d NOEC	1600[2]	OECD 202; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna	Reproduction	21d NOEC	4670[2]	OECD 202; Number of offspring / individual; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna	Number of breeds per female	21d NOEC	7940[2]	OECD 202; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna	Somatic growth rate	21d NOEC	7940[2]	OECD 202; Growth of the length of the first exopodite of the second antenna; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna	Intrinsic population growth rate	21d NOEC	7940[2]	OECD 202; Nominal concentrations	2	Martins et al., 2012[1]

Species	Endpoint & Duration		Effect value (µg/L)	Guideline/Comments	Reliability evaluation	Reference
Daphnia magna	Age at 1st breed	21d NOEC	13 500[2]	OECD 202; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna	Reproduction	21d EC_{50}	11 500[2]	OECD 202; Number of offspring / individual; Nominal concentrations	2	Martins et al., 2012[1]
Daphnia magna (neonates F0)	Reproduction	21d EC_{20}	11 000	OECD 211		Dalla Bona et al., 2015
Daphnia magna (neonates F1)	Reproduction	21d EC_{20}	24 000	OECD 211		Dalla Bona et al., 2015
Daphnia magna (neonates F0)	Survival	21d NOEC	15 000	OECD 211		Dalla Bona et al., 2015
Daphnia magna (neonates F1)	Survival	21d NOEC	15 000	OECD 211		Dalla Bona et al., 2015
Mollusca						
Potamopyrgus antipodarum	Reproduction	56d NOEC	0.4[2]	Total number of embryos; Nominal concentrations	3	Nentwig, 2008[1]
Potamopyrgus antipodarum	Reproduction	56d NOEC	≥0.8[2]	Total number of embryos; Nominal concentrations	3	Nentwig, 2008[1]
Fish						
Danio rerio (juvenile)	Growth rate (weight)	28d NOEC	<780	Nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Lebistes reticulatus (juvenile)	Growth rate (weight)	28d NOEC	780	Nominal concentrations	3	Załeska-Radziwiłł et al., 2011[1]
Cyprinus carpio (eggs)	Growth	33d NOEC	500	OECD 210; Increased growth		Zivna et al., 2016
Cyprinus carpio (eggs)	Growth	33d LOEC (NOEC)	3000 (1000)	OECD 210; Reduced growth		Zivna et al., 2016
Cyprinus carpio (eggs)	Development	33d LOEC	1	OECD 210		Zivna et al., 2016
Denio rerio (juveniles)	Growth rate	28d NOEC	>3000	OECD 215		Plhalova et al., 2014

1 = Data and reliability evaluations from Swiss Ecotox Centre (2013). 2 = Ciprofloxacin-HCl used as test substance, factor of 0.9 was used to convert to ciprofloxacin.

Table S5. MIC distribution of ciprofloxacin collected from EUCAST. Concentrations are given in mg/L. ECOFF = epidemiological cut-off values. Dist = Distribution. Obs. = Observations.

Species	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF	Dist.	Obs.
Acinetobacter baumannii	0	0	12	7	27	224	833	865	410	174	109	262	118	176	311	360	127	193	3	1.0	115	4211
Acinetobacter calcoaceticus	0	0	0	0	7	17	33	31	24	21	6	2	0	0	0	8	1	0	0	1.0	2	150
Acinetobacter lwoffii	0	0	0	3	12	59	73	47	21	8	11	3	1	0	15	9	0	0	0	1.0	2	262
Actinomyces israelii	0	0	0	0	0	0	0	0	1	1	0	0	5	45	35	10	1	0	0	ND	1	98
Bacteroides fragilis	0	0	0	0	0	0	0	0	1	4	48	58	37	65	27	3	5	0	0	ND	2	248
Burkholderia cepacia	0	0	0	0	1	4	2	4	11	5	10	15	10	11	4	1	3	0	0	ND	3	81
Campylobacter coli	0	0	0	0	27	592	2176	1240	256	36	6	58	322	482	178	54	2	0	0	0.5	44	5429
Campylobacter jejuni	0	0	0	9	250	3692	4121	975	166	38	18	149	1380	811	377	195	31	0	0	0.5	43	12212
Citrobacter braakii	0	0	2	3	2	3	0	0	0	0	0	0	0	0	2	0	0	0	0	ND	1	12
Citrobacter freundii	0	0	1	8	3	1	2	4	0	1	3	0	0	0	1	0	0	0	0	ND	1	24
Citrobacter koseri	0	0	5	6	3	1	0	0	0	0	1	0	0	0	0	0	0	0	0	ND	1	16
Citrobacter spp	0	5	68	103	366	96	54	26	30	20	18	10	91	1	1	0	0	0	0	0.125	8	889
Clostridium difficile	0	0	0	0	0	0	0	0	0	0	0	0	79	143	13	63	59	171	0	ND	3	528
Clostridium perfringens	0	0	0	0	0	1	2	12	4	2	1	1	0	0	0	0	0	0	0	ND	1	23
Corynebacterium amycolatum	0	0	0	0	1	0	0	0	0	0	0	0	0	0	11	0	0	0	0	ND	1	12
Corynebacterium jeikeium	0	0	0	0	0	0	0	1	0	0	0	0	1	0	10	0	0	0	0	ND	1	12
Corynebacterium pseudodiphtheriticum	0	0	1	0	0	1	1	3	1	0	0	4	0	0	0	0	0	0	0	ND	1	11
Corynebacterium striatum	0	0	0	0	0	0	2	1	0	0	0	0	1	1	23	0	0	0	0	ND	2	28
Corynebacterium urealyticum	0	0	0	0	0	0	0	1	0	1	0	0	0	2	8	0	0	0	0	ND	1	12
Enterobacter aerogenes	0	0	52	150	244	112	96	32	49	47	53	26	43	46	45	217	3	13	0	0.125	48	1228
Enterobacter agglomerans	0	0	1	15	29	3	2	1	3	0	0	0	0	0	0	0	0	0	0	ND	1	54
Enterobacter cloacae	236	423	320	374	365	199	84	90	73	57	21	33	22	23	12	7	10	2	3	0.125	52	2354
Enterococcus faecalis	0	0	0	2	9	3	17	76	680	2105	637	120	53	232	341	593	239	27	180	4.0	16	5314
Enterococcus faecium	0	0	0	0	0	0	4	124	573	926	921	791	170	25	56	198	35	243	0	4.0	25	4066
Escherichia coli	14	189	3967	7300	1576	613	566	599	196	113	55	131	263	236	565	168	85	59	7	0.064	55	16702
Haemophilus	27	577	6081	5080	891	54	21	9	8	9	8	15	6	3	5	0	0	0	0	0.064	22	12794

Species	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF	Dist.	Obs.
influenzae																						
Haemophilus parainfluenzae	0	0	74	111	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.064	1	203
Hafnia alvei	0	0	10	17	27	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0.125	2	60
Helicobacter pylori	5	7	5	51	228	712	1268	769	157	32	27	78	93	81	427	0	0	0	0	0.5	2	3940
Klebsiella oxytoca	0	0	192	553	389	156	106	50	47	45	37	56	27	22	25	12	1	1	0	0.125	54	1719
Klebsiella pneumoniae	0	5	246	874	1925	946	539	315	251	138	100	86	72	60	149	116	38	30	15	0.125	71	5905
Listeria monocytogenes	0	0	0	0	0	0	0	1	28	82	21	9	0	0	0	0	0	0	0	ND	3	141
Moraxella catarrhalis	0	0	24	944	6978	2666	470	25	21	5	3	3	0	0	0	0	0	0	0	0.125	15	11139
Morganella morganii	0	15	78	182	45	9	6	1	2	9	8	4	4	2	4	3	0	3	0	0.125	8	375
Neisseria gonorrhoeae	3093	2012	871	311	150	73	101	158	206	261	568	683	711	366	980	46	6	39	0	0.016	24	10635
Neisseria meningitidis	68	1408	409	5	0	5	6	4	0	0	0	0	0	0	0	0	0	0	0	0.016	16	1905
Pasteurella multocida	0	9	61	135	17	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0.064	6	227
Proteus mirabilis	0	1	54	325	1206	276	219	42	50	85	116	66	24	14	24	20	7	7	1	0.064	12	2537
Proteus vulgaris	0	0	7	28	40	8	9	1	5	0	0	0	0	2	1	0	0	0	0	0.064	3	101
Providencia stuartii	0	0	0	3	3	1	5	0	0	1	4	7	1	1	3	1	2	1	0	ND	2	33
Pseudomonas aeruginosa	0	0	19	42	535	3046	9340	4559	3234	1882	1501	876	928	516	499	720	137	28	105	0.5	82	27967
Serratia liquefaciens	0	0	0	3	3	2	4	0	0	0	0	0	0	0	0	1	0	0	0	ND	1	13
Serratia marcescens	0	0	6	8	67	221	302	57	52	85	82	49	25	7	8	7	2	0	0	ND	6	978
Shigella flexneri	0	0	1	10	10	1	0	0	2	1	0	0	2	3	1	4	0	0	0	ND	1	35
Shigella sonnei	0	0	2	16	6	0	0	4	2	0	0	1	2	0	0	1	0	0	0	ND	1	34
Staphylococcus aureus	0	0	3	16	121	785	5421	13547	14679	2972	862	247	1961	425	260	449	383	111	40	1.0	67	42282
Staphylococcus auricularis	0	0	0	0	0	1	8	5	3	3	1	1	0	0	0	0	0	0	0	ND	1	22
Staphylococcus capitis	0	0	0	0	3	7	52	154	33	9	3	4	20	0	0	1	0	0	0	1.0	3	286
Staphylococcus cohnii	0	0	0	0	0	0	3	4	6	1	1	0	0	0	0	0	0	0	0	1.0	1	15
Staphylococcus epidermidis	0	0	0	5	36	143	1228	2582	680	202	234	535	2831	73	149	256	58	15	0	1.0	8	9027
Staphylococcus haemolyticus	0	0	3	0	1	19	131	196	52	11	27	9	39	117	17	34	26	96	0	1.0	6	778
Staphylococcus hominis	0	0	1	0	2	31	170	75	36	34	30	42	182	0	2	1	0	0	0	1.0	3	606

Species	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF	Dist.	Obs.
Staphylococcus hyicus	0	0	0	0	0	0	164	34	10	1	2	1	2	0	0	0	0	0	0	ND	2	214
Staphylococcus intermedius	0	0	0	0	0	1	3	5	5	8	2	1	0	0	0	0	0	0	0	1.0	1	25
Staphylococcus lugdunensis	0	0	0	0	3	3	18	29	11	4	5	1	0	1	1	0	0	0	0	1.0	3	76
Staphylococcus saprophyticus	0	0	0	0	0	9	31	185	1111	35	10	4	1	2	2	2	0	0	0	1.0	4	1392
Staphylococcus simulans	0	0	0	0	0	6	11	10	12	4	0	0	0	0	0	0	0	0	0	1.0	1	43
Staphylococcus warneri	0	0	0	0	0	2	21	68	30	1	0	1	5	0	0	0	0	0	0	1.0	2	128
Stenotrophomonas maltophilia	0	0	1	1	3	26	11	35	150	576	886	529	276	140	20	7	0	0	146	ND	15	2807
Streptococcus agalactiae	0	0	2	0	0	4	30	106	1559	1511	279	14	2	9	2	0	0	0	193	2.0	12	3711
Streptococcus anginosus	0	0	0	0	0	1	6	6	48	62	26	11	0	2	5	0	1	6	0	ND	6	174
Streptococcus constellatus	0	0	0	0	0	0	4	27	51	14	3	0	0	0	0	0	0	0	0	ND	2	99
Streptococcus intermedius	0	0	0	0	0	0	0	8	19	30	16	0	0	0	0	0	0	0	0	2.0	2	73
Streptococcus mitis	0	0	0	0	1	1	1	9	38	106	171	82	5	0	0	1	0	0	0	4.0	2	415
Streptococcus oralis	0	0	0	0	0	0	0	0	0	0	30	64	35	12	3	1	0	0	0	ND	3	145
Streptococcus pneumoniae	0	0	8	13	26	32	129	1558	11160	42781	15822	1328	299	100	153	100	10	3	1	2.0	50	73523
Streptococcus pyogenes	0	0	0	2	3	4	54	3710	6962	967	855	75	7	1	5	0	0	0	234	1.0	14	12879
Streptococcus salivarius	0	0	0	0	1	1	0	2	29	41	12	1	0	0	0	0	0	0	0	ND	1	87
Streptococcus sanguinis	0	0	0	0	0	0	1	3	23	65	33	2	0	0	0	0	0	0	0	ND	1	127
Streptococcus uberis	0	0	0	0	0	0	0	14	51	30	2	0	0	0	0	0	0	0	0	ND	2	97
Yersinia enterocolitica	0	0	3	14	143	145	0	1	9	0	0	0	0	0	0	0	0	0	0	0.25	4	315

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