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Species-related risk assessment of antibiotics using the probability distribution of long-term toxicity data as weighting function: a case study

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Abstract Urban areas are among the main sources which release antibiotics into the environment. The fate of antibiotics during their passage through the human body, the sewer system and the waste water treatment processes can be estimated and used for ecological risk assessment. The present approach deals with the possibility of addressing the ecological impact on individual trophic levels using a probability function to attenuate the classical PNEC approach. The species sensitivity distribution (SSD) is based on available long-term toxicity data and was fitted using the Hill-equation. The species-related toxicity threshold was merged with the slope characteristics gathered from SSD to express the risk probability of each species level. The results for algae and crustaceans show that azithromycin, clarithromycin and ciprofloxacin contribute the highest risk portions to the risk index (RI). The determined RI for fish was found to be below the threshold value of 1 and thus no risk is expected for this species.

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1 Introduction

Chemical substances which are released into the aquatic environment carry the potential to cause harm to a variety of organisms and compartments (Cao et al. 2009; Guo et al. 2013). The impairment of a few established organisms in natural ecosystems can severely disrupt the sensitive food web which requires a proper characterisation and control of the according substances. In this context, the European Commission offers a widely accepted guideline (Technical Guidance Document on Risk Assessment—TGD) on how to assess the environmental risk of chemicals in e.g. water bodies (EU 2003). This guideline is based on the identification of Predicted No-Effect Concentration (PNEC), which is put into relation with a Predicted or Measured Environmental Concentration (PEC or MEC) to define the resulting risk (Fahd et al. 2014). According to the TGD high risk is defined as PEC/PNEC ratios above 1, while the Swedish Environmental Classification of Pharmaceuticals considers 10 as threshold ratio for a substance provoking high risk to the environment (fass.se 2014). The difference is equivocal and leads to a drawback of the risk rating system. There are no procedures proposed on how risk values above 1 (hereinafter referring to the ranking system of the TGD) are supposed to be dealt with. Ranking the risk-substances according to the species level which they actually affect is a promising adjustment of the widely accepted methodology of chemical risk assessment and will be proposed in this work.

Considering a reliable determination of PNEC, the procedure allows a good estimation of the overall risk to

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water bodies. The risk linearly depends on PEC which is a necessary assumption, but does not reflect the typical dose–effect-relationship (Cao et al. 2009) for organisms. Usually a logarithmic sigmoidal shaped function is applied to express the relationship in this context (Chevre et al. 2008). Using the linear instead of the sigmoidal dependency between risk and substance concentration most probably leads to a vast overestimation of risk since it is not ecologically justified. Hence, the present approach will evaluate the use of the species sensitivity distribution (SSD), proposed by TGD (EU 2003), to approximate a sigmoidal shape as correction function for the calculated risk. With the aid of the Hill coefficients D_H and m, substances with a lack of toxicity information can be linked to a corresponding dose–effect relationship, or SSD, according to their mode of action and potency. This procedure allows the trophic-level-based risk assessment for those substances which significantly contribute to the overall risk, in terms of emitted amounts, but offer little or no information on ecotoxicology.

Antibiotics were chosen to demonstrate the concept of the proposed risk assessment approach. This group of pharmaceuticals is of great scientific and environmental interest due to their resistance-promoting potential and ecotoxic effects. At the present, there are some concerns, that the release and spread of antibiotic resistant genes affect the therapeutic potential of antibiotics against human pathogens (Zhang et al. 2009), which greatly decreases their operational area. The crux regarding these concerns is that no reliable methods exist to assess the risk of antibiotic resistances at present, which complicates the evaluation of implications that antibiotic resistances in the environment have for public health. Many investigations have been carried out to gain knowledge on the occurrence and dissemination of antibiotic resistances. However, the current available information on resistances in the environment is still very limited and more data are needed to better understand their development and selection processes (Rizzo et al. 2013). Investigations on resistances against heavy metals and antibiotics show that both types often occur simultaneously (Yamina et al. 2012). Furthermore, heavy metals co-select for antibiotic resistances and hence directly influence their development (Seiler and Berendonk 2012). The introduction of the minimal selection concentration (MSC), which can be several orders of magnitude lower than concentrations causing observable effects to organisms, gives evidence that the selective pressure under very low antibiotic concentrations is high enough to permanently maintain resistances in the ecosystem (Gullberg et al. 2014). This effect progresses with increasing number of compounds (e.g. heavy metals, herbicides) added to the system.

On the other hand, the extensive use of antibiotics has also posed the question of the risk they provoke in

environmental compartments from the chemical point of view. Considering the standard species algae, crustacean and fish, the former is assumed to be the most sensitive in the aquatic food chain, causing the highest effect if being stunt by toxic substances. Comparing toxicological investigations of antibiotics regarding the three species mentioned above, differences according to their sensitivity ranking can be observed. For instance, crustaceans are more susceptible to clarithromycin than algae, on the other hand fish proved to be the most sensible species in presence of trimethoprim (see supplementary material S1). The evaluation of these differences can therefore help to prioritize substances more detailed than it is possible using the standard PNEC-approach (see supplementary material S2).

2 Materials and methods

2.1 Concept

The TGD suggests to perform a SSD to determine the most probabilistic PNEC value. The SSD includes all available long-term toxicity data[1] on different taxonomic groups of organisms. It is assumed that they follow a theoretical distribution function and that each group of organisms tested in the laboratory constitutes a random sample of this distribution. Assuming an adequate amount of data, the PNEC is defined as the 5 percentile of the SSD. An assessment factor of 1–5 is applied and accounts for further uncertainties, e.g. the diversity and representativeness of the included organisms or the adequate coverage of sensible life stages of the organisms. Additionally, the TGD provides guidance on the minimal requirements for SSD in terms of number of taxonomic groups and available NOEC as well as information how multiple data on species are to be considered. Those information state general principles on how to perform extrapolation techniques but are hardly applicable to antibiotics. The majority of substances neither have the variety of taxonomic groups nor the amount of data available to carry out a "proper" risk assessment, in terms of a SSD. Nevertheless, the guideline is widely accepted and offers valuable information on how to deal with available data sets. Hence, the present approach will comply with the basic principles of the guideline.

The general idea is that the SSD specifies the probability of the PNEC to be accurate, depending on the available data. This distribution is substance-specific and hence provides a measure on how sensitive organisms respond to

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[1] No Observable Effect Concentration (NOEC), Lowest Observable Effect Concentration (LOEC), EC10 (Effect Concentration causing 10 % effect) and LC10 (Lethal Concentration causing a lethal effect of 10 %) values were combined in this study to extend the applicable data set.

concentration changes (mode of action). This characteristic will be used to transfer the specific dose–effect relationship to the three classical species algae, crustaceans and fish. Depending on their minimal NOEC value the SSD distribution is going to be adjusted in order to describe each effect probability individually. Subsequently, the probability is used to weight the calculated risk quotient, according to its reliability. A summary of the steps to species-related risk assessment is given in Fig. 1.

The SSDs will be fitted using the HILL-equation (Eq. 1). The parameter m defines the curve's slope and is specific for the relevant mode of action. This assumption was confirmed for herbicides and pharmaceuticals with similar mode of action (Chevre et al. 2006, 2008) and hence can be transferred to the group of antibiotics. D_H expresses the substance potency and is defined as the concentration where PNEC-probability amounts to 50 % (see Chevre et al. 2006; supplementary material S3 for equation conversion). The PEC was calculated as outlined in 2.3.3.

The determination of all SSD was not carried out for antibiotics with less than 3 available effect concentrations, instead the SSD character of other antibiotics with the same mode of action was adopted. This approximation was necessary for azithromycin, clindamycin, roxithromycin and doxycycline which share the same mode of action with clarithromycin (block of protein biosynthesis) whereas the former also share the same point of action. Cefuroxime was the only cephalosporine with toxicity data and provides the slope characteristics for cefaclor, cefadroxil and cefixime. Penicilline V belongs to the same beta-lactam group as amoxicillin and will be described by the latter. All antibiotics investigated in this study are summarized in Table 1.

$$f_{AB} = \frac{1}{\left(\frac{D_H}{PEC_{AB}}\right)^m + 1} \qquad (1)$$

The parameter m (also referred to as slope) has significant impact on the share in risk an antibiotic (AB) contributes. Low data availability might lead to wrong conclusions of slopes, a drawback which needs to be addressed to. Therefore, the influence of m was evaluated by globally setting different m values from 0.1 to 3.0 to all substances (hereinafter addressed to as SSDm), neglecting the SSD determined for each substance. The global use of m implies identical modes of action for every antibiotic class. This assumption can be accepted at this point since sufficient data do not exist to prove the opposite for environmental organisms.

In this approach the distribution characteristic determined from all species is assumed to be valid for single species, too. Even if the use of individual values for m, which defines the shape of the characteristic distribution, seems to be the appropriate procedure to characterize the risk on the species level, it is not practicable in this case. The factor influences the curve progression and describes the organism's sensitivity to the substance. The higher the chosen or approximated m is, the steeper the curve becomes. Comparing two dose–effect distributions, one with low potency and flat curve progression (high D_H, low m) and the other having the reverse characteristics, the low potency curve exhibits higher probability values at lower concentrations and exceeds the high potency distribution, in terms of calculated effect probability. This instance may be right for some antibiotics, but cannot be assumed in the first place without having the adequate data available. For this reason identical parameter m are applied to all species level under investigation.

Fig. 1 Flowchart of the proposed methodology using the probability distribution of long-term toxicity data as weighting function for environmental risk assessment on the species level for single substances
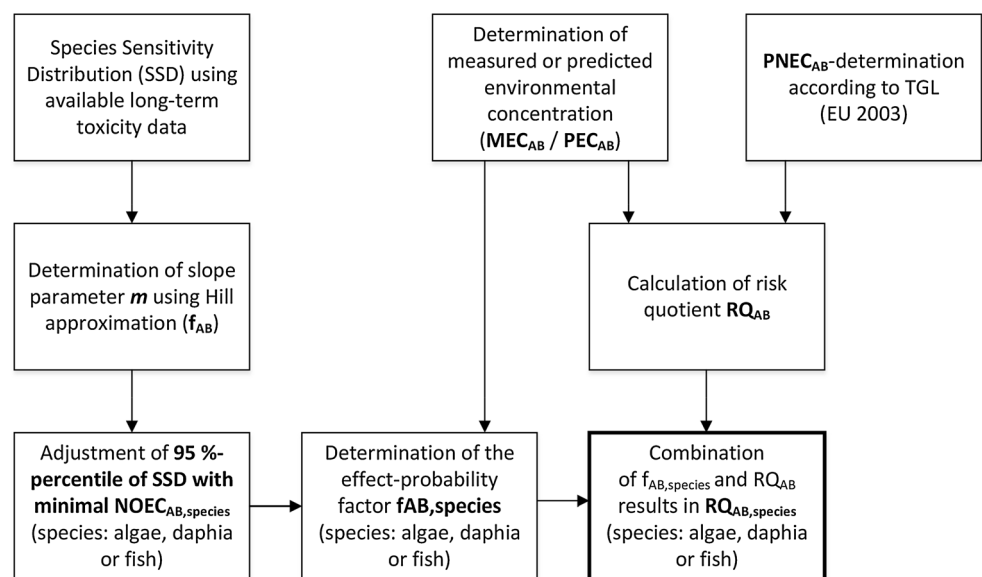
Table 1 Prescription data of investigated antibiotics

Antibiotic	Therapeutic class	Prescription in WWTP catchment (2005–2011) (kg/a)	Share in group (%)	Total group coverage (%)
Cefadroxil	Cephalosporine (1. generation)	10 ± 3	3	91
Cefuroxime	Cephalosporine (2. generation)	302 ± 58	78	
Cefaclor	Cephalosporine (2. generation)	25 ± 8	6	
Cefixime	Cephalosporine (3. generation)	14 ± 5	4	
Clarithromycin	Macrolide	85 ± 26	23	82
Azithromycin	Macrolide	24 ± 9	6	
Roxithromycin	Macrolide	24 ± 11	6	
Clindamycin	Lincosamide	176 ± 45	47	
Amoxicillin	Penicillin	243 ± 41	40	88
Penicillin V	Penicillin	286 ± 39	48	
Ciprofloxacin	Fluoroquinolone	140 ± 9	55	86
Levofloxacin	Fluoroquinolone	80 ± 10	31	
Sulfamethoxazole	Sulfonamide	207 ± 27	100	
Doxycyclin	Tetracycline	44 ± 8	82	
Trimethoprim	Diaminopyrimidine	42 ± 6	98	

The overall risk quotient (RQ) will be calculated using the wastewater treatment plant's effluent concentration of each antibiotic (PEC), which was generated from prescription data (see 0). Subsequently, the RQ is weighted using the effect-probability factor ($f_{AB,species}$) arising from SSD adjustment (see 2.2 and Eq. 2), according to the considered species and antibiotic (Eq. 4). For the calculation of the risk index (RI) the method of concentration addition is applied (Eq. 3) (EPA 2000). The RI will serve as standardization method to calculate the relative impact of single antibiotics on the overall risk of the species level. As a result, this procedure, complying with TGD guidelines, extends the generally accepted risk assessment methodology to a species-based risk ranking within the aquatic ecosystem.

$$f_{AB,species} = \frac{1}{\left(\frac{1-0.95}{0.95}\right) \cdot \left(\frac{NOEC_{AB,species}}{PEC_{AB}}\right)^m + 1} \quad (2)$$

$$RI_{species} = \sum RQ_{AB,species} \quad (3)$$

PNEC values applied in Eq. 4 were taken from literature and compared with the results from the SSD of each antibiotic. A brief overview regarding the selection of PNEC is presented in the supplementary material S4.

2.2 Derivation of species-based risk assessment

Only for a few substances an adequate data set is available to reasonably carry out a SSD. Sulfamethoxazole is a well investigated compound for which reason thus it was chosen to exemplify the involved steps of species-based risk assessment.

At first, the Hill curve is fitted to the data points of all available species to calculate the dose-probability as well as the potency of each substance (see Fig. 2). The next step involves merging the minimal effect concentration of each species with the slope characteristics gathered from SSD (and SSDm). According to the 5 % percentile for PNEC-estimation, the 95 % percentile of SSD_{SUL} is adjusted to the corresponding minimal $NOEC_{AB,species}$ value of each species (=5 %-ile of Hill curve, dashed grey curve in Fig. 2). However, at this concentration the $RQ_{AB,species}$ nearly equals the RQ determined from classical PNEC calculation. From this point, reduced concentrations lead to lower $f_{AB,species}$ and attenuated $RQ_{AB,species}$.

The shifted probability distribution was calculated using Eq. 2. Considering the individual potency of each species, the formula is applied to the calculated effluent concentration in order to determine the probabilistic factor for each RQ.

$$RQ_{AB,species} = \frac{PEC_{AB}}{PNEC_{AB}} \cdot f_{AB,species} \quad (4)$$

It is assumed, that the probability of each species to be affected by an antibiotic depends on the mode of action and hence is identical within one group of antibiotics (for groups see Table 1). Therefore, missing species-related NOEC-values were replenished using the probability distribution of the antibiotic in question in combination with a NOEC-probability of another, better investigated antibiotic within the same group. For example, the NOEC probability for algae of ciprofloxacin (3.00 µg/l) constitutes 24 % which in turn assigns an NOEC probability for algae of

Fig. 2 Example for fitting the SSD to Hill-equation using sulfamethoxazole (SUL) as an example
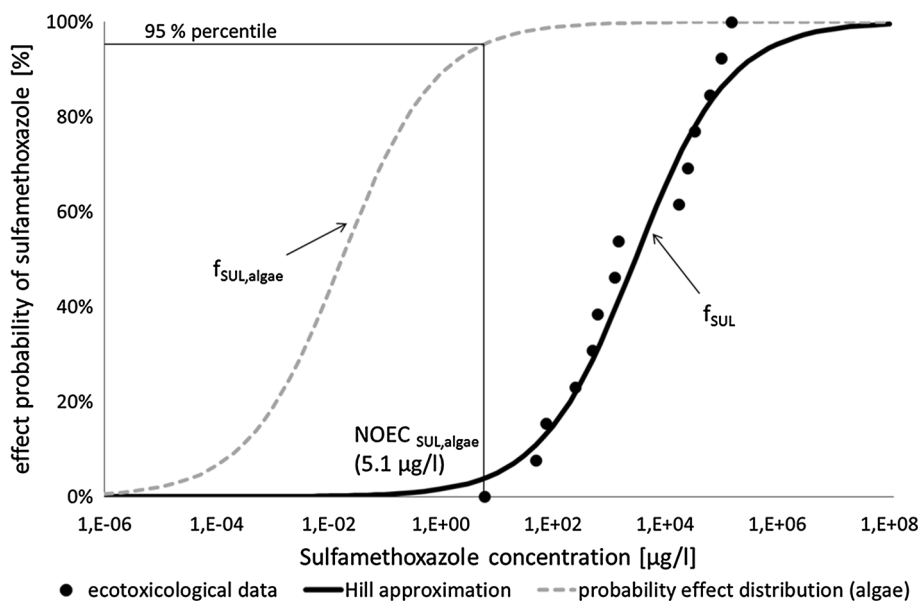
84.96 µg/l for levofloxacin, regarding its specific probability distribution (see Table 3).

No crustacean and fish data are available for antibiotics belonging to the group of fluoroquinolones. Hence, the probability of crustacean and fish to be affected by levofloxacin and ciprofloxacin was divided into three and set to 67 and 100 %, respectively. This implies that fish (highest effect concentration) are more tolerant to fluoroquinolones than crustaceans, which in turn are more tolerant than algae (lowest effect concentration). Yielded effect concentrations are given in Table 3.

2.3 Input data and scope of the study

2.3.1 Substances

Prescription data of antibiotics from 2005 to 2011 were provided by the statutory health insurance company AOK PLUS which insures about 41 % of the people living in Dresden, Germany. The dataset covers only ambulant drug prescription. Additionally, data on the consumption of antibiotics were provided by the three major hospitals covering about 65 % of hospital beds available in the catchment area of the wastewater treatment plant (WWTP). In Table 1 the 15 most prescribed antibiotics in Dresden which were chosen for this study are listed. Ofloxacin and levofloxacin are racemats and considered as one substance.

The sewer system of the WWTP Dresden-Kaditz has a length of 1,700 km and collects the wastewater of five cities (Dresden, Freital, Heidenau, Pirna and eastern Radebeul) and some bordering municipalities. Presently, the wastewater of approximately 650,000 inhabitants is

being treated, whereof 80 % live in Dresden. It is assumed that the prescription data of AOK-insured people in Dresden can be extrapolated to the entire catchment area of the WWTP and to all respective inhabitants. The theory of antibiotic person equivalents was examined comparing the calculated amount of the total catchment area with the bordering municipalities of the catchment area. The ratio of five investigated antibiotics with seasonal and non-seasonal influence yielded 15–30 % of the total input to the total catchment area (see Fig. 3). To cover the entire catchment area, the mean share of the five presented antibiotics (24 %) will be used for extrapolation purposes of remaining antibiotics.

Furthermore, it is accepted that insurants of private health companies, which hold a share of 10 % of the total inhabitants, receive the same type and amount of antibiotics as those being statutorily insured. The approximated net number of commuters of about 40,000 (6 % of the catchment's population) will not be considered since antibiotic taking usually involves a sick certificate.

2.3.2 Scope of the case study

Estimating the input load of antibiotics into the aquatic environment using prescription or production data is an accepted method for the environmental risk assessment of chemicals (Besse and Garric 2008; Ortiz de Garcia et al. 2013). Hence, this methodology was applied to estimate the environmental hazard induced by antibiotics, including their specific human excretion information and elimination rate during wastewater treatment (see Table 2). Apart from that stated above, there is a variety of processes and factors

Fig. 3 Bordering municipalities' input share in the entire catchment area of the WWTP (data set: weekly prescription from 2005 to 2011)
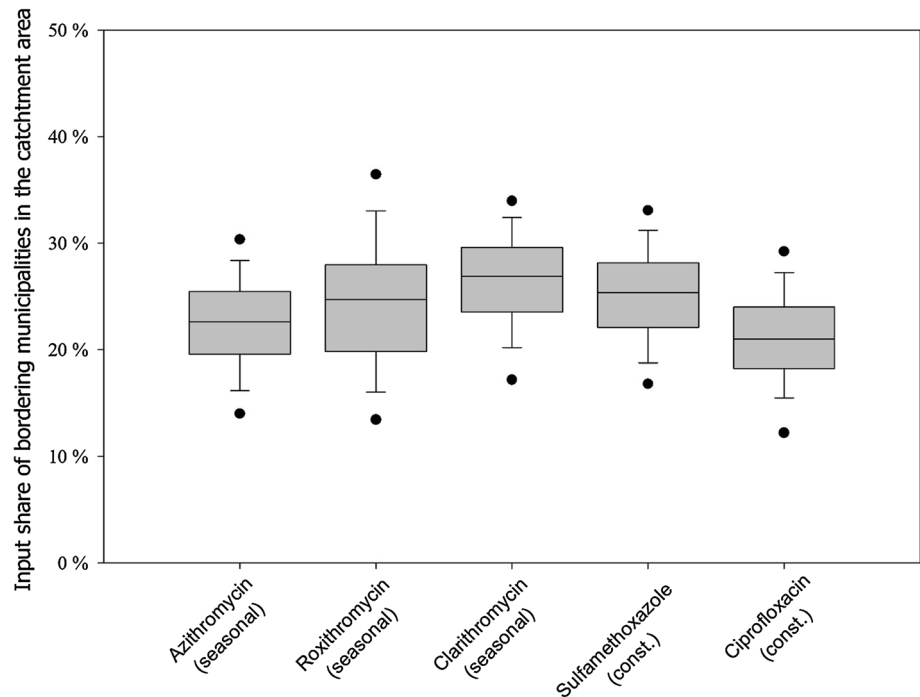
in natural water bodies that have a potential influence on the assessment of antibiotics and can lead to different risk characteristics than determined by the general methodology. For differentiation purposes an overview of possible interaction processes will be briefly presented in the following in order to define the boundary conditions for the case study under investigation.

After their release into natural water bodies antibiotics are prone to a variety of processes which lead to a change of concentration and hence a change of toxicity. Depending on the actual situation the mixing of the effluent and the receiving stream usually results in lower antibiotic concentrations. Regarding the exemplifying case under investigation, the mean daily low flow of the river Elbe was determined to 102 m^3/s (DWSO 2014) and indicates a mean dilution of the wastewater effluent (1.8 m^3/s) of about 1/57, if complete mixing is assumed. In contrast, due to the slow transverse mixing between the effluent and the receiving river a dilution of the former is not achieved for several flow kilometers and substance concentrations remain higher than the complete mixing calculation suggests. The level of transverse mixing depends on the receiving stream's characteristics (width, flow velocity, degree of meandering) and an estimation is difficult to carry out without applying appropriate mixing zone models (Jirka et al. 2004).

Despite the fact that the main part of antibiotics with favorable adsorption characteristics are eliminated during wastewater treatment (excess sludge), sediment–water interactions (sorption) still contribute to an alteration of antibiotic concentrations in natural water bodies (Zhou and

Broodbank 2014). Besides, the processes hydrolysis and photolysis are considered as further elimination pathways, leading to transformation or complete mineralization. Hereby, temperature, pH and inorganic matter in the receiving river play a crucial role concerning the influence that sorption (Gu and Karthikeyan 2008; Zhang et al. 2014), hydrolysis (Bialk-Bielinska et al. 2012; Kang et al. 2012; Mitchell et al. 2014) and photolysis (Kümmerer 2009; Sirtori et al. 2012; Wang and Lin 2012) have regarding the overall elimination of antibiotics. In case of incomplete mineralization, transformation products technically become a part of the risk assessment. Nevertheless, the classical risk assessment only addresses the toxicity of the parent compounds under investigation, neglecting transformation products from technical (wastewater treatment) or natural (e.g. photolysis) elimination of antibiotics. Considering the fact that some transformation products can exhibit a higher toxicity compared to their parent substance (El Najjar et al. 2013; Iskender et al. 2007; Sirtori et al. 2012; Wang and Lin 2012) makes the necessity of including those substances in the risk assessment evident. However, the high number of possible and partly unknown compounds causes the inclusion of transformation products to be per se incomplete and hence difficult to carry out and evaluate.

As shown above, the dilution and elimination in natural water bodies as well as the transformation pathways are difficult to assess in a complete manner due to the variety of substances, processes and changing conditions (pH, inorganic matter etc.) along the flow path. In order to demonstrate the concept of the proposed methodology the

Table 2 Excretion and elimination rate of antibiotics under investigation

Antibiotic	Excretion rate	Elimination during wastewater treatment
Amoxicillin	60–85 % (mean: 72.5 %) (Aktories et al. 2009; Martindale 1993)	99 % (Watkinson et al. 2009)
Azithromycin	67,4 % (Sandoz 2009)[a]	0 % (Goebel et al. 2007)
Cefaclor	53 % (Lode et al. 1979)	100 % (Watkinson et al. 2007) (used in study: 99 %)
Cefadroxil	88 % (Pfeffer et al. 1977)	50 % (assumption, no data available)
Cefixime	18 % (Brittain et al. 1985)	50 % (assumption, no data available)
Cefuroxime	50 % (ODDB 2014)	60 % (unpublished data)
Ciprofloxacin	40 % (urine), 15 % (faeces) (sum: 55 %) (Aktories et al. 2009; Vancebryan et al. 1990)	66 % (Li and Zhang 2011)
Clarithromycin	60 % (Hirsch et al. 1999)	0 % (Goebel et al. 2007)
Clindamycin	10–35 % (mean: 22.5 %) (Pharma 2012; Still et al. 2006)	0 % (Watkinson et al. 2007)
Doxycyclin	22–70 % (mean 46 %) (Hirsch et al. 1999; Pharma 2008)	67 % (Yang et al. 2005)
Levofloxacin	74.9–85.9 % (mean: 80.4 %) (Wagenlehner et al. 2006)	58 % (Xiao et al. 2008)
Penicillin V	40 % (Hirsch et al. 1999)	99 % (unpublished data)
Roxithromycin	60 % (Hirsch et al. 1999)	0 % (Goebel et al. 2007)
Sulfamethoxazole	15–25 % (mean: 20 %) (Hirsch et al. 1999; Martindale 1993)	62 % (Li and Zhang 2011)
Trimethoprim	40–60 % (mean: 50 %) (Martindale 1993)	3 % (Lindberg et al. 2005)

[a] Using Eq. 5 with $f_{A,AB}$ = 37 % and $f_{M,AB}$ = 88 %

scope of this study focuses on the single parent substances (no transformation products, no synergistic effects from antibiotic mixtures) in the urban catchment and the respective WWTP. The manifold processes in the receiving river Elbe, which cannot be adequately included without using appropriate hydrological and material flow models, will not be considered.

2.3.3 Antibiotic's fate within the urban catchment and the WWTP

As described in the previous section, the case study focuses on the antibiotic flow starting with the consumption and ending at the outlet of the WWTP. After the consumption a partial elimination of the parent substance takes place during its passage through the human body. They are partly adsorbed by the gastric mucosa and/or gut and may be prone to metabolizing processes. The content that was not adsorbed is assumed to be excreted unchanged. The excreted ratio of the parent compound (E_{AB}) can be calculated using Eq. 5, where $f_{A,AB}$ characterises antibiotic-dependent the adsorption ratio in the human body. The parameter $f_{M,AB}$ determines the share of the respective antibiotic in the adsorbed fraction that is prone to metabolism, which in turn decreases the amount of the parent substance in the faeces. The necessary information are usually provided by the pharmaceutical industry (see Table 2).

$$E_{AB} = (1 - f_{A,AB}) + f_{A,AB} \cdot (1 - f_{M,AB}) \qquad (5)$$

Many anthropogenic substances pass through the sewer system and WWTP before they are released into the environment. During their passage they are prone to adsorption and degradation processes which differ significantly among substances (Liu et al. 2013). In case of antibiotics, most of the penicillins are easily biodegradable due to cleavage of the beta-lactam ring and rarely found in the effluent of WWTP (Watkinson et al. 2007). Cephalosporines, being a sub-group of the latter, show higher persistence in raw wastewater and the treatment process. The macrolides azithromycin, clarithromycin and roxithromycin show highly variable elimination rates from − 45 to 55 % (Goebel et al. 2007). In order to consider the uncertainties regarding macrolide's reduction in WWTP, the elimination rate is thus set to 0 % for this study. Tetracyclines and fluoroquinolones have strong adsorption capabilities. Their main removal route is expected to be excess sludge removal (Golet et al. 2003) which influences the fate in sewer systems as well. There is no evidence that tetracyclines are biodegradable (Kim et al. 2005). Sulfonamides are partly removed in waste water treatment whereas sorption to sludge is irrelevant (Watkinson et al. 2007; Yang et al. 2005). A summary of the excretion and elimination rates applied in this study can be seen in Table 2.

Considering the elimination pathways before the substance's discharge into the receiving river the PEC can be calculated using the following equation:

$$PEC_{AB} = \frac{\left(\frac{P_{AOK,Dresden}}{0.41 \cdot (1.00 - 0.24)} + \frac{P_{hospitals,Dresden}}{0.65}\right) \cdot (1 - \eta_{Elimination})}{Q_{WWTP}}$$

(6)

with the ambulant prescription data of Dresden ($P_{AOK,Dresden}$), the available hospital prescription ($P_{hospitals,Dresden}$), the daily WWTP outflow of Dresden-Kaditz (Q_{WWTP}), the elimination during the wastewater treatment ($\eta_{Elimination}$), the share of insured inhabitants (41 %), the share of bordering municipalities (24 %) and the share of hospital beds covered by the hospital prescription (65 %). In the region of Dresden agriculture plays a minor role and antibiotic inputs originating from veterinary use are not expected.

3 Results and discussion

3.1 Species-related toxicity thresholds

In Table 3, toxicity threshold values related to algae, crustacean and fish are listed for the antibiotics under investigation. The values are either taken from literature (see supplementary material S1) or estimated from the substitutes approach. The most potent antibiotics regarding toxicity towards algae are amoxicillin and penicillin V with effect concentrations of 1.17 µg/l. Furthermore, ciprofloxacin (3.00 µg/l), the macrolides azithromycin, clarithromycin and roxithromycin (11.54–25.00 µg/l) as well as sulfamethoxazole (5.90 µg/l) are also to be considered to provoke effects on the algae level. The species of crustaceans show to be responsive to azithromycin and clarithromycin (4.40 and 4.70 µg/l) plus ciprofloxacin, clindamycin, doxycycline, roxithromycin and sulfamethoxazole (30.20–250.00 µg/l). The most effective antibiotics regarding fish toxicity are trimethoprim (157.00 µg/l) and levofloxacin (937.13 µg/l). Considering the results above macrolides and ciprofloxacin are the most potent substances regarding their effect on the lower trophic levels in this study.

Antibiotics are purposefully designed to decrease the abundance of bacteria in the human body. Hence, it is not surprising that some toxicological investigations using environmental bacteria as test organisms also show considerable effects caused by some antimicrobial agents. Levofloxacin is being effective towards vibrio fisheri at 1.99 µg/l and ciprofloxacin affects pseudomonas putida at 4.90 µg/l. Hartmann et al. (1998) used a genotoxicity assay to investigate the effects of clarithromycin onto bacteria and determined a LOEC of 5.00 µg/l. Bacteria showed a similar sensitivity against toxicity like algae, however, differences in potency ranking can be seen. Unfortunately,

the available data sets are not sufficient to carry out a separate bacteria-related risk assessment for the entire spectrum of antibiotics which are covered in this study. At this juncture, it has to be assumed that bacteria, which should be the most susceptible organisms, show similar effects like algae, even if few toxicological investigations seem to suggest otherwise.

3.2 Evaluation of risk distribution using individual slopes

The RI_{algae} nearly completely fails to fulfil the quality criteria of <1. Only at the beginning of 2006, a RI_{algae} below 1 can be determined (see supplementary material S5). Concluding from calculated values, a permanent risk on the algae level emanates from antibiotics in the effluent of WWTP. Taking a closer look onto the contributing substances, the following conclusions can be drawn. The antibiotics showing the highest risk potency do not significantly contribute to the overall risk for algae originating from the WWTPs effluent. Beta-lactams are well biodegradable and the expected effluent concentration of 0.02–0.03 µg/l is rather low. Their contribution to the RI_{algae} is about 1–2 %. The effluent concentration of ciprofloxacin is about 0.42 µg/l and causes the highest risk share in RI_{algae} of about 59 % ($RQ_{CIP,algae} = 2.84$). Azithromycin and clarithromycin have a contribution to the total risk of 15 % each. Sulfamethoxazole accounts for a minor share of around 6 %, only.

It is proposed to define the substances which contribute more than 80–90 % to the overall risk of the most sensitive species, in this case algae, as first-order risk substances. Algae play a crucial role in aquatic ecosystems and many edible species are being consumed by zooplankton (e.g. crustaceans), which in turn are the basis for fish ranking higher in the food chain. In order to prioritize measures to reduce the substance-induced risk on aquatic ecosystems the proposed ranking gives advice on which substances should be focused on. Accordingly, ciprofloxacin, azithromycin and clarithromycin constitute first-order risk antibiotics, whereas ciprofloxacin clearly provokes the highest risk among them.

It can be seen that risk indices for crustaceans ($RI_{crustacean}$) is nearly as high as RI_{algae} (on average 92 % of RI_{algae}, $R^2 = 0.95$, p < 0.01, see Figs. 5, S5), which is surprising since a significantly higher sensibility for algae was expected. Still, the composition of $RI_{crustacean}$ is somewhat different (see Fig. 4) from that of RI_{algae}. As expected from the species-related thresholds, azithromycin (35 %), along with ciprofloxacin (35 %) hold the highest share in $RI_{crustacean}$. The RQ of clarithromycin constitutes on average 1.20 and amounts 26 % of $RI_{crustacean}$. Additionally, sulfamethoxazole contributes an average $RQ_{SUL,crustacean}$ of 0.12 which,

Table 3 summary of the fitting parameters and toxicity threshold for each species

Antibiotic	D_H (µg/l)	Slope (m) [−]	species-related toxicity threshold		
			Algae (µg/l)	Crustacean (µg/l)	Fish (µg/l)
Amoxicillin	222,250	0.87	1.17	2,300,000	182,700
Azithromycin	35[a]	1.18[a]	11.54	4.4	84,000
Cefaclor	201,658[b]	1.74[b]	76,000[b]	83,100[b]	1,000,000[b]
Cefadroxil	2,016,58[b]	1.74[b]	76,000[b]	831,000[b]	1,000,000[b]
Cefixim	201,658[b]	1.74[b]	76,000[b]	831,000[b]	1,000,000[b]
Cefuroxime	201,658	1.74	76,000	831,000	1,000,000
Ciprofloxacin	13	0.78	3	30.2	4,535.06
Clarithromycin	35	1.18	11.54	4.7	1,000,000
Clindamycin	256[c]	1.83[c]	81.1[a]	42.07[a]	329,020.05[a]
Doxycyclin	256[c]	1.83[c]	81.1[a]	42.07[a]	329,020.05[a]
Levofloxacin	137	2.39	84.96[e]	181.17	937.13
Penicillin V	222,250[d]	0.87[d]	1.17[d]	2,300,000[d]	182,700[d]
Roxithromycin	477	2.48	25	183.88	62,383.17
Sulfamethoxazole	1,945	0.54	5.9	250	33,848.57
Trimethoprim	11,394	0.87	3,100	13,000	157

[a] Adopted from clarithromycin

[b] Adopted from cefuroxime

[c] Adopted from azithromycin and clarithromycin by averaging

[d] Adopted from amoxicillin

[e] Adopted from ciprofloxacin using probability distribution
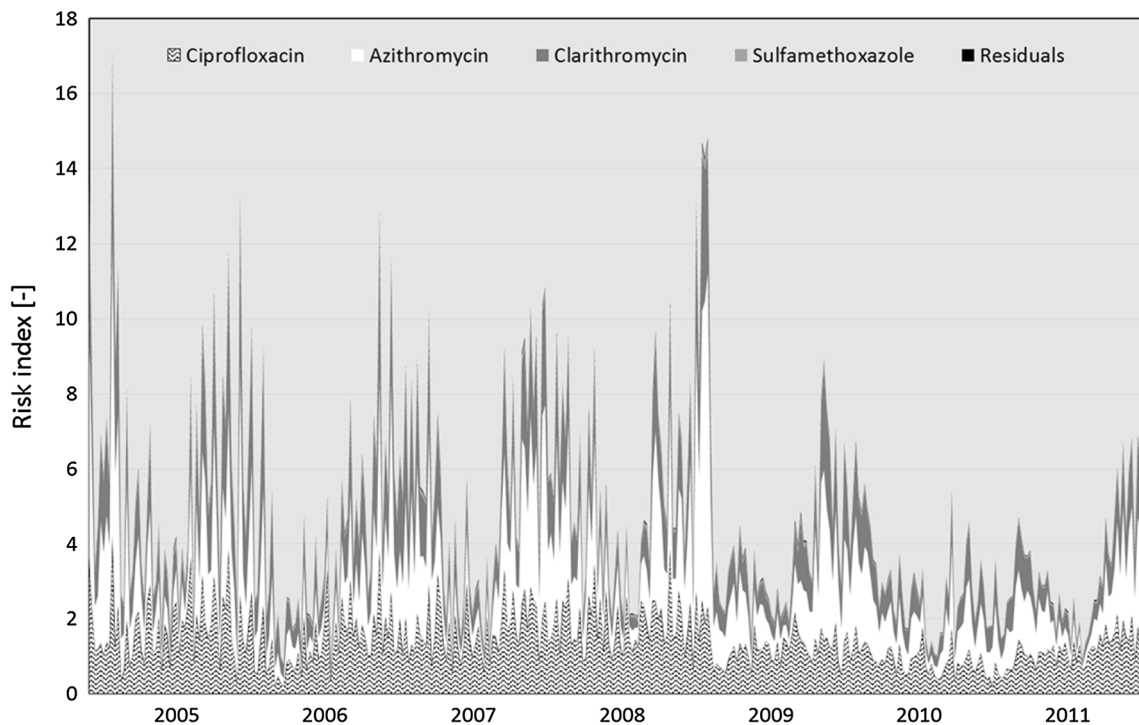
Fig. 4 Risk index of the species crustacean ($RI_{crustacean}$)—remaining RQ_{AB} are summed up and displayed as residuals

depending on the season, corresponds to a proportion between 1 and 6 % of $RI_{crustacean}$. The substances which induce the risk to crustaceans are the same as those for algae, even though their proportions are different. Crustaceans are more susceptible to macrolides which in turn compensates for the higher sensitivity of RI_{algae} to ciprofloxacin. The RI_{fish} amounts up to 0.20 (see supplementary material S5) and

consists mainly of RQ_{CIP} and RQ_{SUL}. The index does not exceed the threshold value of 1 which leads to the conclusion of an acceptable risk for fish.

Using the proposed sigmoidal probability distribution rather than a linear relationship between concentration and estimated risk leads to a mean attenuation of the classic PNEC-approach ($RI_{classic}$) of about 70 % for algae and

crustaceans. The attenuation for RI_{fish} is even higher and reduces the risk index below the threshold value of 1. The relationship between the species-related and the classical RI are shown in Fig. 5.

3.3 Evaluation of risk distribution using global slopes

The probability distributions used in this approach are influenced by the number and quantity of investigated species. Owing to circumstances of randomly investigated organisms an unequal distribution of available data regarding the trophic levels is possible. In order to overcome, or assess, these uncertainties and evaluate the robustness of the present approach, the parameter m of the Hill equation was set constant for all antibiotics and globally diversified to estimate its influence on risk proportions, depending on its dimension.

In general, a similar distribution pattern can be observed for algae and crustaceans, compared to the approach using individual slopes. Regarding algae, ciprofloxacin becomes massively dominant above a slope of 0.5 and reaches a share in total risk of over 90 % at a slope of 3 (see Fig. 6a). Azithromycin and clarithromycin contribute a minor part to $RI_{m,algae}$, which complies with the results of individual distribution curves for antibiotics.

Azithromycin and clarithromycin show to be the most potent antibiotics for crustaceans (see Fig. 6b). Below a slope of 1.5 the former contributes the largest risk

proportion. Increasing m up to 3 causes the influence of clarithromycin to rise further, while azithromycin becomes less important. Compared with the approach using individual slopes, ciprofloxacin affects the risk to a lesser extent.

Evaluating the risk proportion of fish (see Fig. 6c), it becomes apparent that levofloxacin and trimethoprim dominate the determined risk. Ciprofloxacin, which contributed between 70 and 90 % to the overall RI_{fish} using individual slopes, reaches a proportion of around 30 % at $m = 0.5$ and is further decreasing with higher m-values. The reason for the observed differences are to be found in the comparatively low species-related toxicity thresholds of levofloxacin and trimethoprim. Ciprofloxacin's potency is lower by factors 5 (levofloxacin) and 28 (trimethoprim) which leads to minor RQ_{CIP} at identical m-values.

The slope m also has a stake in the dimension of RI_{algae}, $RI_{crustacean}$ and RI_{fish}. A slope of zero is almost matching the average $RI_{classic}$ (24.54), only reduced by a factor of 0.95 (setting m to zero in Eq. 2; Fig. 6d). High slope values describe a faster response to concentration changes, which increases the effect probability considerably. But this is only the case, if calculated effluent concentrations are close or above the species-related toxicity thresholds. Substantially lower effluent concentrations lead to probability factors close to zero and, subsequently, to decreasing RI. RI_{fish} hits the threshold value of 1 at a slope of around 0.6, RI_{algae} and $RI_{crustacean}$ reach the threshold line at around 2.3
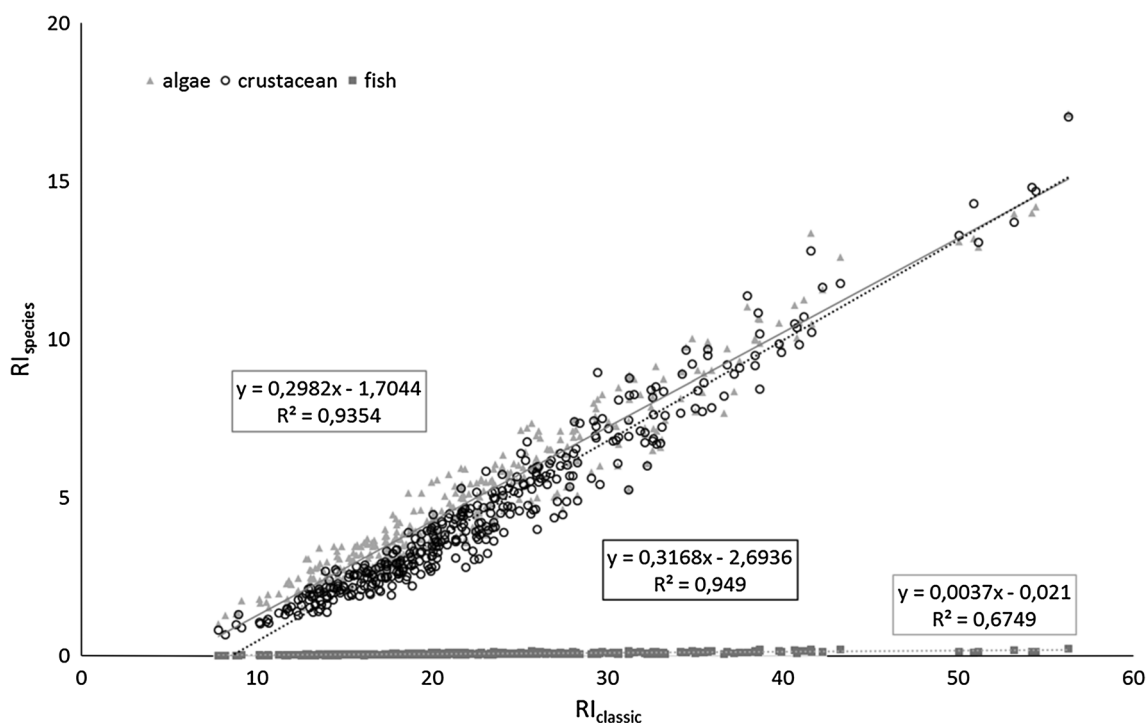
Fig. 5 Species-related $RI_{species}$ versus classical PNEC-derived $RI_{classic}$
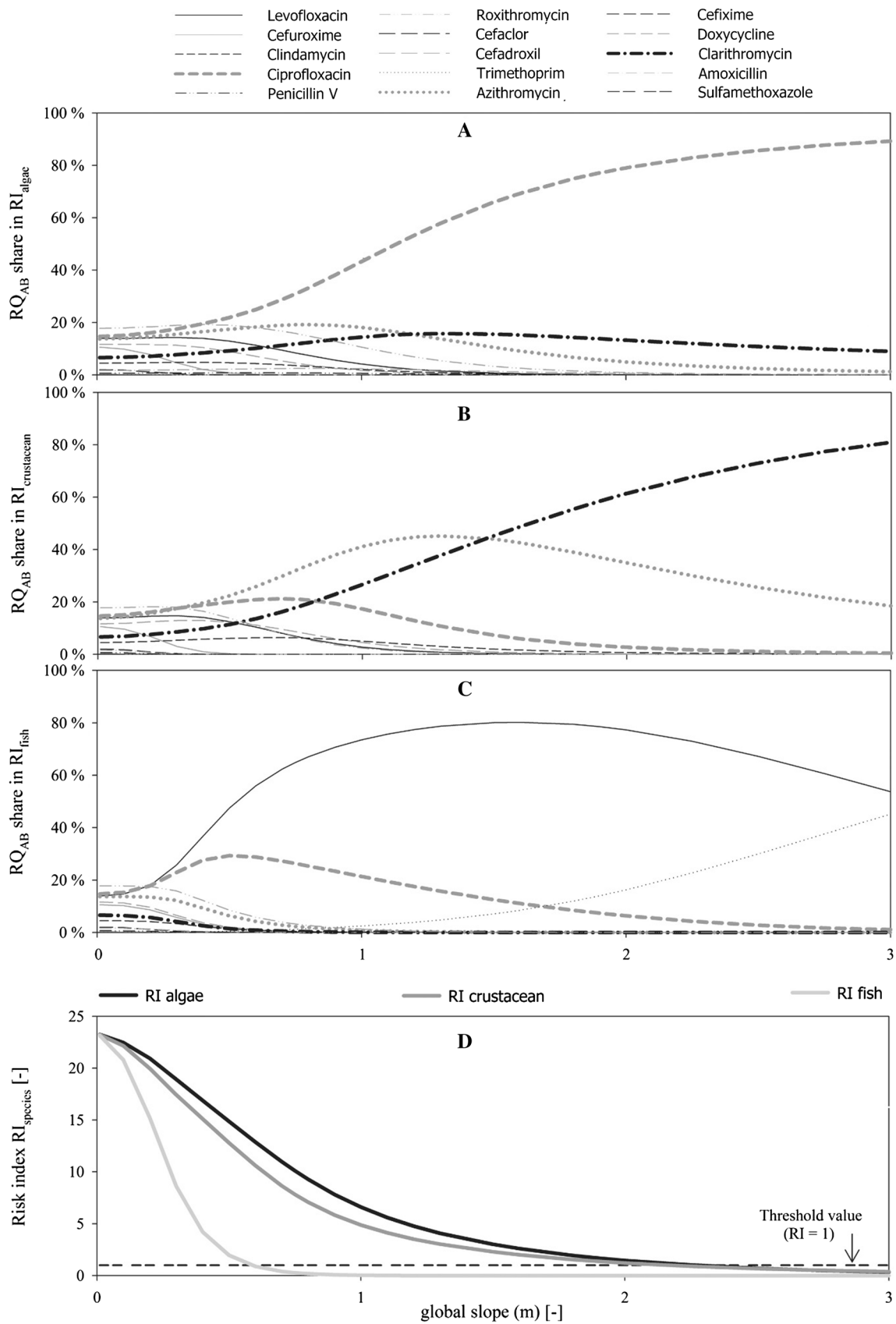
Fig. 6 Risk proportions using global *m*-values (**a** algae; **b** crustacean; **c** fish; **d** influence on RI$_{species}$)

and 2.0, respectively (see Fig. 6d). Due to the interpretation that antibiotics do not constitute a risk to the environment above these *m*-values, a further investigation of the risk proportions will not be necessary. Chevre et al. (2008) studied the sensitivity distribution of herbicides, organophosphates and beta-blockers. The lowest slope calculated from toxicity data of the pharmaceutical group beta-blockers was 0.6 which is assumed to represent the lower limit of this investigation, too (see supplementary material S3 for formula conversion). Consequently, the assessed risk towards fish is low which corresponds well with the approach applying individual slopes. Within the range of reasonable *m*-values the risk assessed for the species algae and crustaceans is high and shows similar risk distributions of antibiotics, independently of the approach used.

4 Conclusions and summary

The introduced approach addresses two drawbacks of the PNEC-based risk assessment. Firstly, the linear relationship between concentration and effect can be corrected using the probability distribution as weighting function, also referred to as SSD. The resulting attenuated risk quotient RQ reflects the quality of the data set as well as the effect range which is defined through the variety of test organisms. Secondly, a differentiated risk assessment on the species level delivers insight into the real impact potential of antibiotics. It was shown, that algae are the most sensitive among the investigated species, while environmental bacteria might prove to be even more susceptible to antibiotics. The latter need to be intensively investigated in the future in order provide a reliable data basis and to give a justified answer to this question.

The combined approach of using a sigmoidal probability distribution on the species level leads to strongly attenuated risk indices. RI_{algae} and $RI_{crustacean}$ are 70 % lower than the RI determined using the classical PNEC approach. RI_{fish} was even decreased below the threshold value and thus does not indicate a risk for this species.

The application of both global and individual *m*-values is suitable to estimate the contribution to the overall risk from each substance. The first-order priority substances, i.e. those that hold the highest share in the overall risk at the most sensitive species-level in the food chain, can be identified using either global or individual slopes for the probability distribution. The knowledge of first-order priority substances is a solid step into bottom-up risk management from the chemical's point of view, concentrating on the substances which are the most hazardous to the aquatic environment. In case of lower-tier organisms being affected by the substances under investigation a damage propagation among species-levels, which results in the weakening of higher-tier organisms, must be taken into account. Hereby, it must be considered that standard testing procedures cover the effect measuring using indicators like the uptake/elimination of test chemicals, growth rate or mobility which result in the classical toxicologic endpoints, e.g. EC_{10}. Hence, no conclusion can be drawn regarding the kind of effect that influences the respective organism.

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