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Antibiotic concentrations in European rivers

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Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers

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Abstract

This study evaluated the potential concentrations of four antibiotics: ciprofloxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erythromycin (ERY) throughout the rivers of Europe. This involved reviewing national consumption rates together with assessing excretion and sewage treatment removal rates. From this information, it was possible to construct best, expected and worst case scenarios for the discharge of these antibiotics into rivers. Consumption data showed surprising variations, up to 200-fold in the popularity of different antibiotics across different European nations. Using the water resources model GWAVA which has a spatial resolution of approximately 6 x 9 km, river water concentrations throughout Europe were predicted based on 31-year climate data. The modelled antibiotic concentrations were within the range of measurements reported previously in European effluents and rivers. With the expected scenario, the predicted annual-average antibiotic concentrations ranged between 0 and 10 ng/L for 90 % by length of surface waters. In the worst case scenario concentrations could reach between 0.1 and 1 μg/L at the most exposed locations . As both predicted and observed sewage effluent concentrations were below reported effect levels for the most sensitive aquatic wildlife, no direct toxicity in rivers is expected. Predicted river concentrations for CIP and ERY were closest to effect levels in wildlife, followed by SUF which was 2-3 orders of magnitude lower. TRI appeared to be of the least concern with around 6 orders of magnitude difference between predicted and effect levels. However, mixture toxicity may elevate this risk and antibiotic levels of 0.1-1 μg/L in hotspots may contribute to local environmental antibiotic resistance in microorganisms.

Key words: ciprofloxacin, sulfamethoxazole, trimethoprim, erythromycin, risk, rivers, environment, toxicity

1. Introduction

The discharge of pharmaceuticals in wastewater into the aquatic environment has been a source of concern in scientific circles for more than a decade (Daughton and Ternes, 1999; Verlicchi et al., 2012) and is now appearing on the agenda of European legislators. This was highlighted by a proposal from the European

Commission to add some pharmaceuticals to the list of priority substances (COM(2011)876). The current Article 8c of 2013/39/EU (Priority Substances Directive) requires the Commission to develop a strategic approach to pharmaceuticals and water pollution. There are approximately 3,000 pharmaceuticals in general use today (Rand-Weaver et al., 2013) so it is difficult to decide which represent the greatest threat to aquatic wildlife. A variety of approaches can be found in the literature to help us decide where to focus our attention (Guillen et al., 2012) including pharmaceutical sales, detection in the environment, risk of exceeding an effect concentration and persistence. Many of these reviews have ranked antibiotics as amongst the pharmaceuticals of greatest concern for the aquatic environment (Al Aukidy et al., 2014; Besse and Garric, 2008; Christensen et al., 2009; Dong et al., 2013; Kumar and Xagoraraki, 2010; Ortiz de Garcia et al., 2013).

Antibiotics have played a major role in improving human health and supporting livestock production since World War II. As they are not completely metabolised in the body, widespread discharge into the aquatic environment from both domestic and agricultural sources occurs. Amongst river organisms, it would seem that blue-green algae (prokaryotes), are the most sensitive with direct toxicity at a few μg/L (Ando et al., 2007; Halling-Sorensen, 2000). Thus, antibiotics might reduce algal biodiversity. Another concern with the discharge of antibiotics is the potential development of resistant bacteria, even at low concentrations (Gullberg et al., 2011). Although, antibiotic resistance in the environment may be the result of excreted bacteria from patients or animals themselves, it may have been stimulated in response to the antibiotic discharge. Some have argued that this is happening already in rivers downstream of sewage treatment plants (STPs) or intensive agriculture (Moore et al., 2010; Winkworth, 2013).

This study examined four different antibiotics; ciprofloxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erythromycin (ERY). CIP is part of the fluoroquinolone group of antibiotics, which became widely used from 1990, it targets the DNA gyrase of bacteria and so inhibits cell replication (Hooper et al., 1987). SUF belongs to the sulphonamide group which inhibits an enzyme involved in the synthesis of tetrahydrafolic acid (part of the thymidine metabolic pathway in DNA synthesis). TRI acts by targeting another enzyme

involved in the tetrahydrafolic acid pathway and so SUF and TRI have often been used together in therapy since the late 1960s (Burchall, 1973; Seydel et al., 1972). ERY has been used since the 1950s and is part of the macrolide group of antibiotics which is believed to act on the 70S RNA ribosome thereby preventing transfer RNA from moving and so halting peptide synthesis (Igarashi et al., 1969).

Ciprofloxacin, SUF, TRI and ERY have been identified as antibiotics of particular concern by scientists in the aquatic environments of the UK, Denmark, Sweden, France, Spain, USA and Worldwide (Besse and Garric, 2008; Castensson et al., 2009; Christensen et al., 2009; Dong et al., 2013; Hughes et al., 2013; Jones et al., 2002; Kaplan, 2013; Lienert et al., 2007; Ortiz de Garcia et al., 2013). The high risk ranking of these particular four antibiotics relative to others has been linked to their consumption, discharge, persistence and toxic properties. Apart from human patients, agriculture accounts for a large amount of antibiotic consumption and of the four antibiotics selected, TRI appears to be the most popular in veterinary practice in Europe (Kools et al., 2008). However, the route to rivers from this source is unpredictable, thus this study only focused on discharge from domestic sources for which the route to surface waters is relatively well understood and therefore predictable.

The overall objective of this study was to examine how close European river concentrations of four of the antibiotics of particular concern are to reported acute toxicity levels. Some river measurements of these antibiotics exist, however, isolated grab samples can give a misleading impression of exposures and hence risk. Geographic information system (GIS) based river water quality modelling provides an alternative approach to spot sampling through providing a wide range of predicted values associated with geography and hydrological natural variability. A review of the strengths and weaknesses of these two approaches for polar contaminants has been made before, but both methods combined can support one another to give greater confidence in risk assessment (Johnson et al., 2008). In this study the Global Water Availability Assessment model (GWAVA) (Meigh et al., 1999) was used in a water quality mode to predict antibiotic concentrations throughout the rivers of Europe. The following objectives were addressed:

- To examine different European national per capita antibiotic consumption rates

- To compare the range of predicted concentrations in effluents with those reported in the literature,
- To predict surface water concentrations throughout Europe using a spatially explicit model,
- To compare national predicted river concentrations against published measured concentrations
- To examine whether current predicted concentrations might exceed those which are acutely toxic to algae, the most sensitive aquatic species?

2. Methods

2.1. Method introduction

The process begins with collecting information on national consumption which is converted to a per capita rate. Then information is gathered on excretion rates of the parent compound followed by a review of removal rates of the parent compound in sewage treatment. This information can be used to predict effluent concentrations for different countries using assumptions on wastewater discharge per capita. The research involved using ranges of key words on popular academic search engines to acquire literature and then examining the references in that literature to widen the net. Peer- reviewed data were preferred, unless these were absent or excellent quality publicly available information was present. This does not guarantee all the best literature is found, nevertheless the assumptions and consequent rates can be tested against measured values. To predict concentrations in real river water situations, the rate information can be imported into GIS-based water quality models. The model will associate the population connected to sewage treatment plants with the pharmaceutical consumption/excretion and sewage removal information previously collected in actual river networks.

2.2. Assessing per capita consumption rates

The first and arguably most important hurdle to overcome in any predictive model aiming to report concentrations of human derived pharmaceuticals in water is obtaining accurate information on consumption (Johnson et al., 2008). If this is incorrect the modelling exercise will fail before it has begun. Reports in the literature can be used to assess a per capita consumption, given the population of the country

105 at that time (Table 1). The consumption data found ranged from as recent as four years to sixteen years ago.

106 However, overall changes in European national antibiotic consumption over time tend to be small as

107 revealed in a survey of use for 1997-2009. For example, over this period there was a 2% increase in the UK,

108 an 8% drop in Spain and a 14% increase in Germany (Adriaenssens et al., 2011).

109

Table 1

National antibiotic drug consumption data (mg/cap/d) collected for ciproflaxacin (CIP), Sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY). The data, originally in g/year, was transformed to mg/cap/day using the national population*[1] at the time of the survey.

Country	**Source**	**CIP use & year (mg/cap/d)**	**SUF use & year (mg/cap/d)**	**TRI use & year (mg/cap/d)**	**ERY use & year (mg/cap/d)**
Germany	(Roig, 2010; ter Laak et al., 2010)	0.471(2006)*[2]	1.786 (2006)	0.405 (2001)	0.704 (2006)
France	(Roig, 2010; ter Laak et al., 2010)	0.562 (2006)	0.770 (2008)	0.906 (2008)	0.822 (1998)
UK	NHS dataset from www.ic.nhs.uk (Boxall et al., 2014; Roig, 2010)	0.331 (2010)	0.049 (2006)	0.597 (2014)	1.82 (2010)
Spain	(Carballa et al., 2008; de Garcia et al., 2013; Roig, 2010)	1.1 (2010)	0.633 (2010)	0.004 (2010)	5.14 (2003)
Poland	(Roig, 2010)	0.345 (2006)	0.468 (2006)	NA*[3]	0.455 (2006)
Austria	(McArdell et al., 2003)	NA	NA	NA	0.342 (1998)
Greece	(Straub, 2013)	NA	NA	0.194 (2003)	NA
Switzerland	(Alder, 2006; Giger et al., 2003; ter Laak et al., 2010)	NA	0.853 (2004)	0.193 (2004)	0.066 (1999)
Sweden	(Alder, 2006; Lindberg et al., 2005)	1.104	0.440 (2005)	NA	NA
European mean value		**0.652**	**0.820**	**0.418**	**1.336**

110 *[1] Historic population information from indexmundi (http://www.indexmundi.com/factbook/countries)
111 which is compiled from CIA World fact book and the IMF world economic outlook 2011
112 *[2]Information from the 1999-2006 period provided in this reference (Roig, 2010)
113 *[3] Information not provided

114 The difference in CIP consumption between nations with available data was just over 3-fold (most popular in

115 Spain), whilst for SUF consumption differed by 35-fold with the highest apparent consumption occurring in

116 Germany. For TRI, consumption differed by 226-fold with the highest consumption occurring in France
117 whilst ERY consumption differed by 78-fold with the highest consumption occurring in Spain (Table 1). For
118 some antibiotics this consumption is not stable throughout the year, with seasonal increases to treat winter
119 respiratory tract infections (Bruyndonckx et al., 2014; Suda et al., 2014).

120 *2.3. Assessing per capita excretion rates and sewage removal rates*

121 The next stage in estimating the domestic load of a pharmaceutical is to ascertain how much of the
122 parent compound is excreted unchanged by the patient. Age, health and co-medication can all influence the
123 percentage excreted. It is therefore important to survey as much literature as possible on excretion rates to
124 discover the range and find a mean or median value. Similarly, variations in sewage treatment performance
125 can influence pharmaceutical removal rates in treatment. Information on the proportion of these drugs
126 excreted as parent compounds was limited for CIP but more abundant for the other compounds (Table S1).
127 The biggest variations in excretion were associated with ERY (Table S1). There is a large amount of
128 information on how much of these drugs is removed in sewage treatment but the data can vary
129 considerably, for example, ERY removal data ranged from 0 to79% (Table S2).

Table 2

Summary of loss rates used in the modelling for the antibiotics following receipt of national consumption rates. Expected is the median whilst best and worst are the extremes reported in the literature

Losses	CIP losses	SUF losses	TRI losses	ERY losses
Expected excretion (%)	35	18	46	17
Worst case excretion (%)	45	30	60	45
Best case excretion (%)	25	10	43	2.5
Expected removal (%)	76	48	24	36
Worst case removal (%)	61	0	0	0
Best case removal (%)	90	75	69	79

130

131 So the effluent concentrations are predicted by taking the drug consumption per capita for a specific nation
132 less that prevented from being excreted as the free parent compound less that removed in sewage
133 treatment. The effluent concentration (*W*, in ng/L) is derived as follows for a specific nation:

134 $$W = \frac{(C \times E \times (1-R))}{D}$$

135 Where *C* is the substance consumption (ng/cap/d); *E* is the substance amount not excreted (ng/cap/d); *R* is
136 the amount of the drug that is prevented from escaping into sewage effluent (ng/cap/d); and *D* is the
137 volume of wastewater (L/cap/d).

138 *2.4. Scenario analysis*

139 There are uncertainties in the model parameters determining effluent concentrations. Firstly, drug
140 popularity can wax and wane from year to year, and there will be some seasonal trends. Where a national
141 consumption was not known a European average was used. Then there are the uncertainties regarding the
142 amount excreted (Table S1) and removed in sewage treatment (Table S2 and 2). In order to encompass the
143 range of these variables, a series of scenarios were run to cover likely effluent and river concentrations
144 based on the diverging literature values. These scenarios were a best case (low excretion, high sewage
145 removal); a worst case (high excretion, low removal) and an expected case, which used the average values
146 for these parameters. Thus, the best to worst case should encompass the range of possible effluent and
147 river concentrations (Table 2). Seasonal use variation would be difficult to add to the scenarios because
148 different antibiotics might be more, or less, popular in different seasons, which could vary between different
149 countries.

150 When comparing the predicted effluent concentrations calculated using the values from Tables 2, S1
151 and S2 with those reported in the literature for different nations, the expected or best case predictions were
152 frequently closest to reported values (Table 3). Thus, it might be expected that using the worst case scenario
153 would overestimate river concentrations.

Table 3

Comparing expected, worst and best case predicted to measured effluent concentrations for ciproflaxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erythromycin (ERY).

Compound	Country	Scenario	Consumption (mg/cap/d)	Wastewater discharge (L/cap/d) [a]	Predicted effluent conc. (ng/L)	Measured effluent conc. (ng/L)	Reference
CIP	Europe	Expected	0.652	176	311	20-95	(Golet et al., 2003; Lindberg et al., 2005; Miege et al., 2009)
		Worst	0.652	176	844		
		Best	0.652	176	50		
SUF	Spain	Expected	0.633	208	285	438	(Carballa et al., 2004)
		Worst	0.633	208	913		
		Best	0.633	208	76		
	Switzerland	Expected	0.853	328	243	280	(Gobel et al., 2005)
		Worst	0.853	328	778		
		Best	0.853	328	65		
	Sweden	Expected	0.440	205	201	70-233	(Bendz et al., 2005; Lindberg et al., 2005; Wahlberg et al., 2011)
		Worst	0.440	205	644		
		Best	0.440	205	54		
TRI	UK	Expected	0.597	274	762	128-271	(Ashton et al., 2004; Roberts and Thomas, 2006)
		Worst	0.597	274	1307		
		Best	0.597	274	403		
	Switzerland	Expected	0.193	328	205	200	(Gobel et al., 2005)
		Worst	0.193	328	352		
		Best	0.193	328	108		
ERY	UK	Expected	1.82	274	1238	109-832	(Ashton et al., 2004; Gardner et al., 2013a; Roberts and Thomas, 2006)
		Worst	1.82	274	5119		
		Best	1.82	274	96		
	Switzerland	Expected	0.066	328	45	80-150	(Gobel et al., 2005; McArdell et al., 2003)
		Worst	0.066	328	186		
		Best	0.066	328	3		

[a]Based on the water consumption for European countries from Eurostat Water statistics (http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Water_statistics) for the UK wastewater discharge was derived from a study of 20 STPs in the UK (Williams et al., 2012).

2.5. European river water modelling

To estimate concentrations of these antibiotics throughout European surface waters, the spatially-explicit water resources model GWAVA was used in a water quality mode (Dumont et al., 2012; Meigh et al., 1999) as recently used to examine cytotoxic drugs (Johnson et al., 2013). This model considers the location and size of the human population and their association with STPs. The effluents from these STPs are incorporated with other natural and artificial flows together with abstractions into the hydrological model. The hydrology of the model is driven by monthly climate over the period 1970-2000. The per-capita effluent loads used in the model were derived as described previously (Table 2). The model views Europe as a series of grid squares (cells) of approximately 6 x 9 km (5 by 5 Arc minutes). Finding an appropriate spatial scale inevitably involves some compromise between practicality and sufficient precision (Dumont et al.,

168 2008) but for obtaining a pan-European or national impression this scale should yield a representative
169 picture. Where a water course passes through a cell, 372 separate monthly concentrations are estimated,
170 based on the 31 year monthly climate dataset and taking into account the upstream input. GWAVA can also
171 modify the concentrations along the river network by including a water column biodegradation rate.
172 However, in this case the antibiotics were assumed to be conservative once in the river due to limited
173 biodegradation rate information. This assumption that the antibiotics will be conserved in the rivers means
174 the predictions will be precautionary (a bias towards overestimation). Nitrogen, phosphorus, and carbon
175 concentrations predicted by GWAVA have been extensively compared to measured concentration time-series data
176 from across Europe. Whilst concentrations near the mouths of large river basins were found to be generally
177 underestimated, apart from this, there was no systematic over- or underestimation (Dumont et al., 2012).

178 ## 3. Results and discussion

179 *3.1. The impact of different variables*

180 It is possible to review the different factors that could affect the final predictions (Table 4). This
181 approach traces changes in concentration from sewer to effluent by looking at what influence the highest
182 and lowest excretion and sewage treatment removal would have on that concentration expressed as an X-
183 fold difference. The overall difference is the excretion difference multiplied by the sewage treatment
184 removal difference. The greatest variation in consumption across Europe was for TRI and ERY. The biggest
185 range in apparent excretion of the parent molecule was for TRI. There were large uncertainties found in
186 apparent sewage removal of SUF, TRI and ERY. When the impact of the variables on the final effluent
187 concentration are summed up, it is clear that ERY would have the greatest uncertainty (Table 4).
188

Table 4

Summary of the variables and their potential effects on the estimated effluent concentrations for ciproflaxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY).

Drug	Range in consumption across EU	Mean, highest & lowest patient excretion values (%)	Effect on sewage influent conc.	Weighted mean, highest & lowest sewage removal (%)	Effect on sewage effluent conc.	Overall difference between best and worst case

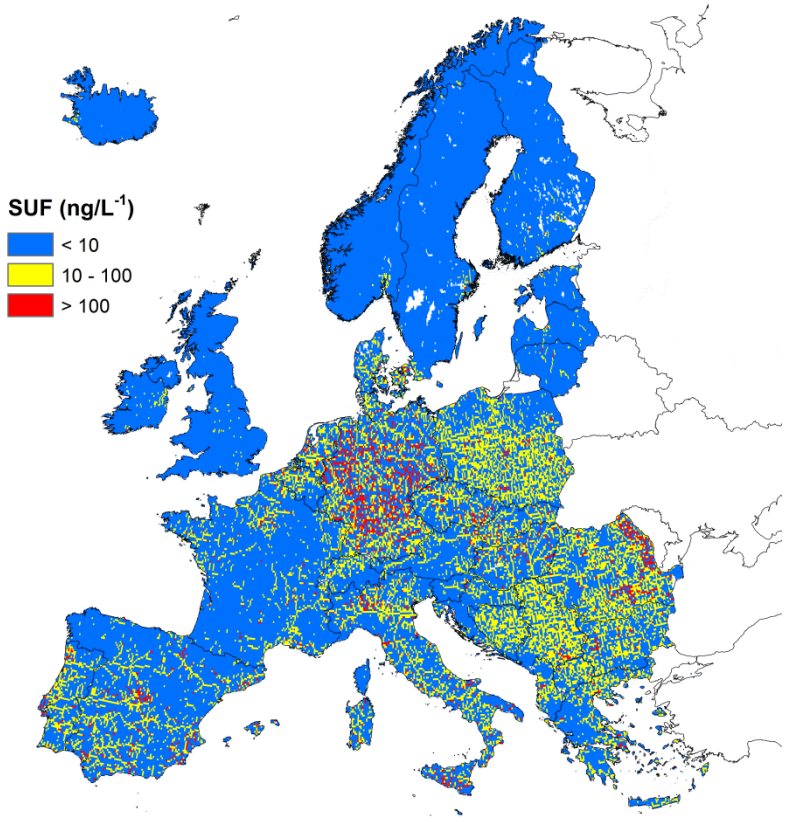
CIP	4.4-fold	35 (25-45)	1.8-fold	76 (70-90)	3-fold	5.4-fold
SUF	36-fold	18 (10-30)	3-fold	48 (0-75)	4-fold	12-fold
TRI	226-fold	46 (43-60)	1.4-fold	16 (0-58)	3.2-fold	4.5-fold
ERY	78-fold	17 (3-45)	15-fold	36 (0-79)	71-fold	1065-fold

189

190 *3.2. Predicted European river antibiotic river concentrations*

191 The distribution of concentrations around Europe is dependent not just on the local geography and
192 hydrology but also on the national drug consumption. The spatial variation in surface water concentrations
193 can be seen in annual average maps for TRI and SUF (Figs. 1 and 2) where the interplay of population
194 distribution, available river dilution and drug popularity can be revealed. It highlights, for example, that TRI
195 is not popular in Spain compared to its European partners (Table 1), whilst SUF is very popular in Germany
196 but much less so in the UK (Table 1). The map also reveals the consistent benefits of relatively low
197 population densities and high available dilution in the Scandinavian countries and others such as Ireland and
198 Greece, as predicted by others (Keller et al., 2014)

199



200

201 **Fig. 1.** Annual average predicted sulfamethoxazole (SUF) concentrations across European surface waters
202 based on **expected case** scenario (lowest excretion rate and highest sewage treatment removal)

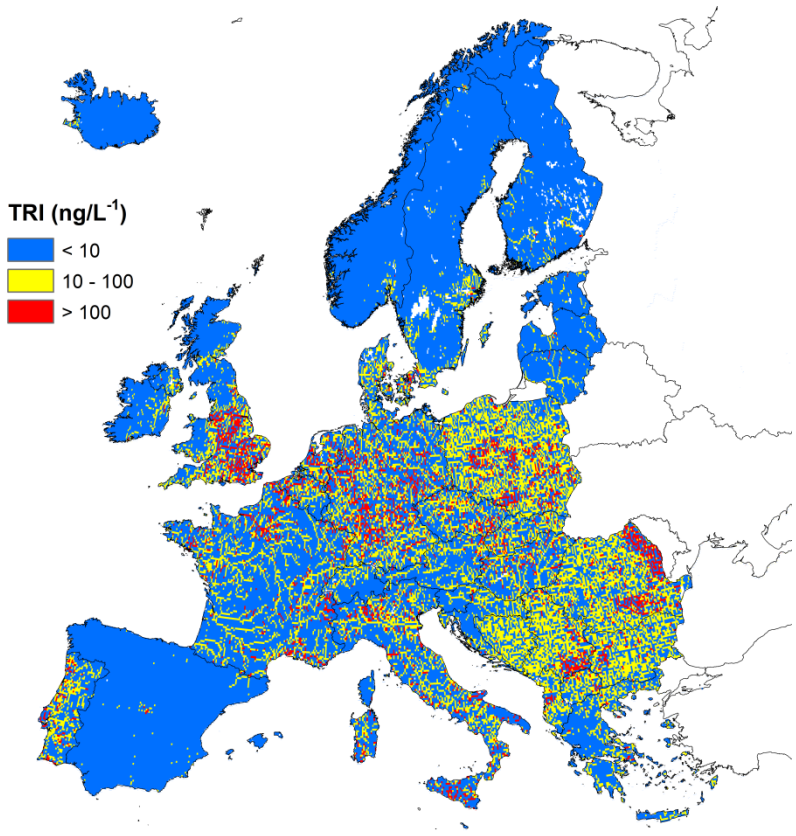


Fig. 2. Predicted annual average trimethoprim (TRI) concentrations across European surface waters based on **expected case** scenario (lowest excretion rate and highest sewage treatment removal)

The complete range of concentrations predicted in the model across Europe can be shown using cumulative frequency curves (Fig 3 and 4) and compared against the lowest reported effect levels for wildlife (Table 5). Each point indicates the percentage of cells (Y -axis) having a concentration exceeding a specific level (X-axis). The curves do not start from 100% as around 25% of European rivers are considered to have no sewage input. With the expected scenario, predicted annual-average antibiotic concentrations range between 0 and 10 ng/L in 90 % by length of surface waters (Fig. 3). It should be clarified that each cell generates 372 results per simulation (based on the 31 years of monthly climate data) and from these a mean, median or percentile can be selected. For the worst case scenario (high excretion and poor removal) and taking the 90%ile for each cell estimated concentrations could reach between 0.1 and 1 μg/L in the most exposed river length for all 4 antibiotics (Fig 4).

3.3. Comparing predicted and measured river antibiotic concentrations

Antibiotics monitored in European rivers fall within the predicted range of concentrations by the modelling (Table 5 and S3), however, the SUF and TRI values tended to fall within the upper percentile of that predicted by the model. Perhaps this should be expected, as many field sampling campaigns looking for these compounds would focus on urbanised areas during lower summer flows. In addition, the use of these products in veterinary medicine might increase river concentrations on occasion (Borriello, 2013; Kools et al., 2008).

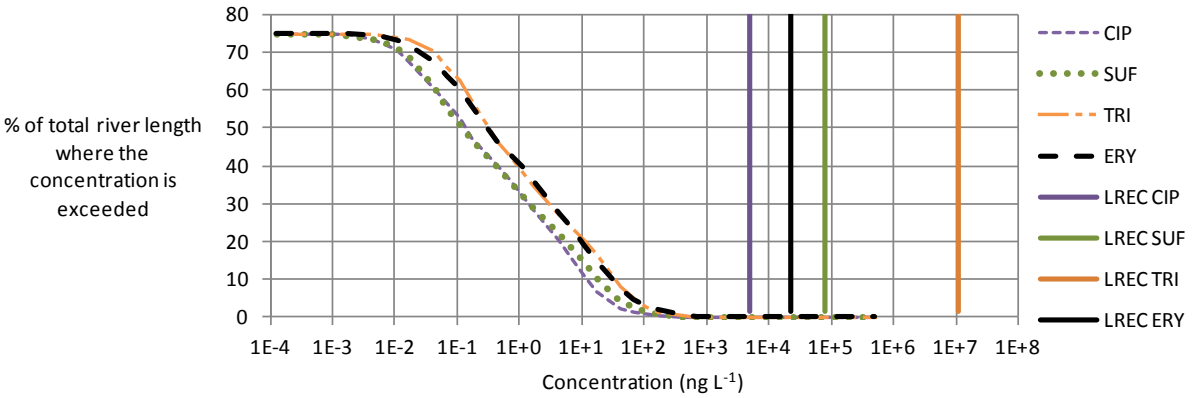


Fig. 3. Predicted **mean** concentrations for ciprofloxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY) in the **expected case scenario** across the whole European continent from the GWAVA model plotted as cumulative frequency curves. Vertical lines labelled LREC are the lowest reported harmful effect concentrations on aquatic wildlife.

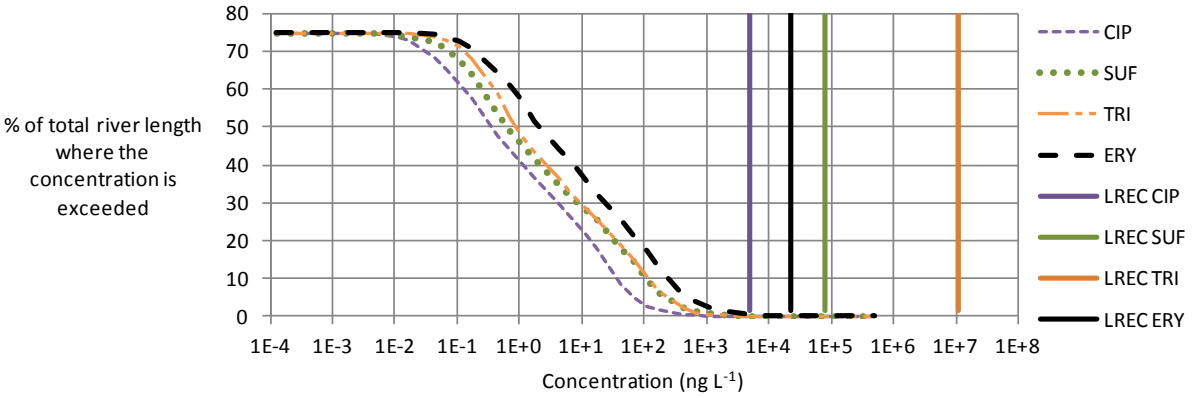


Fig. 4. Predicted **90th** percentile concentrations for ciprofloxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY) in surface water for the **worst case scenario** across the whole European continent from the GWAVA model plotted as cumulative frequency curves.

236

237

238 *3.4. The potential for widespread toxicity from antibiotics in European rivers*

239 These four antibiotics appear to have little or no toxicity to fish or Daphnia species (Table 5). But
240 harmful effects can be seen with the duck weed group which appear to be the most sensitive organisms to
241 SUF. The most sensitive species for the other antibiotics seem to be cyanobacteria followed by green algae
242 with effects observed in the tens of μg/L. Fortunately, it would seem that the antibiotic concentrations
243 measured in regular domestic effluent were all below the median effect levels of the most sensitive species.
244 This indicates that these antibiotics at current levels of consumption are not posing a widespread acute toxic
245 threat to European aquatic wildlife. The CIP and ERY predicted and observed river concentrations appear to
246 be closest to the EC50 levels but still 2 orders of magnitude lower. Trimethoprim appears to be of the least
247 concern, with concentrations predicted to be around 6 orders of magnitude below algal effect levels
248 followed by SUF with 3 orders of magnitude difference. We cannot comment on to what extent such river
249 concentrations might stimulate antibiotic resistance.

Table 5

Comparison of harmful effect concentrations for freshwater organisms reported in the literature for ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin against predicted and observed and river concentrations

Ciprofloxacin						
Toxicity to aquatic wildlife				**Observed and predicted European river concentrations**		
Fish NOEC (μg/L)	**Cladoceran NOEC or fecundity EC50 (μg/L)**	**Duckweed EC50 (μg/L)**	**Cyanobacteria or green algae EC50 (μg/L)**	**Observed river (μg/L)**	**River predicted Worst case 90%ile (μg/L)***	**River predicted Expected mean (μg/L)***
10,000, 100,000	13,000, 60,000	62, 203, 698	8, 10,1,500, 3,000, 18,700	<0.01-0.12	0-0.07	0-0.04
a, b	a, c	b, d, e	a, b, e, f	k, l	This study	This study
Sulfamethoxazole						
Toxicity to aquatic wildlife				**Observed and predicted European river concentrations**		
Fish NOEC	**Cladoceran**	**Duckweed EC50 (μg/L)**	**Cyanobacteria or green algae EC50 (μg/L)**	**Observed river (μg/L)**	**River predicted Worst case 90%ile (μg/L)**	**River predicted Expected mean (μg/L)**
NA*[2]	NA	81	1,530, 112,000, 130,000	<0.01-0.06	0-0.23	0-0.04
NA	NA	d	g, h	l, m, n, o, p, q, r, s, t, u, v, w, x, y, z, aa	This study	This study
Trimethoprim						
Toxicity to aquatic wildlife				**Observed and predicted European river concentrations**		
Fish NOEC (μg/L)	**Cladoceran EC50 (μg/L)**	**Duckweed No effect (μg/L)**	**Cyanobacteria or green algae EC50 (μg/L)**	**Observed river (μg/L)**	**River predicted Worst case 90%ile (μg/L)**	**River predicted Expected mean (μg/L)**
100,000	123,000	>1000	11,000, 80,300, 110,000, 150,000	<0.01-0.18	0-0.23	0-0.06
a	a	d	a, g, i	l, n, o, q, r, s, t, u, x, ab	This study	This study
Erythromycin						
Toxicity to aquatic wildlife				**Observed and predicted European river concentrations**		
Fish	**Cladoceran**	**Duckweed No**	**Cyanobacteria or**	**Observed river**	**River predicted**	**River predicted**

	EC50 (μg/L)	effect (μg/L)	green algae EC50 (μg/L)	(μg/L)	Worst case 90%ile (μg/L)	Expected mean (μg/L)
NA	210,600	>1000	23, 35, 37, 60	<0.01-0.35	0-0.6	0-0.06
NA	j	d	f, g, i	n, q, r, s	This study	This study

a (Halling-Sorensen et al., 2000), b (Robinson et al., 2005), c (Martins et al., 2012), d (Brain et al., 2004), e (Ebert et al., 2011), f (Liu et al., 2011), g (Eguchi et al., 2004), h (Lutzhoft et al., 1999), i (Ando et al., 2007), j (Didelupis et al., 1992), k (Pena et al., 2007), l (Tamtam et al., 2008), m (Banzhaf et al., 2013), n (Dinh et al., 2011), o (Fernandez et al., 2010), p (Garcia-Galan et al., 2011), q (Gros et al., 2007), r (Kasprzyk-Hordern et al., 2007), s (Kasprzyk-Hordern et al., 2008), t (Madureira et al., 2010), u (Martin et al., 2011), v (Nodler et al., 2011), w (Pailler et al., 2009), x (Zhou et al., 2009), y (Houtman et al., 2013), z (Loos et al., 2009), aa (Loos et al., 2010) ab (Boxall et al., 2014)

* The range of modelled values are 5%ile to the 95%ile

*[2] Information not found

4. Conclusions

The modelled antibiotic concentrations were within the range of spot sample measurements reported in European sewage effluent and rivers. Consumption data showed surprising variations, up to 200-fold between the consumption of different antibiotics in European nations. These findings reveal for example that analytical chemists would be much more likely to find TRI in German effluents and rivers than in Spanish ones since consumption is apparently 100-fold higher. Given that predicted CIP and ERY antibiotic river concentrations were only 2-fold below known effect levels for algae, this study endorses the listing of these antibiotics as pharmaceuticals of concern in reviews on the topic (Besse and Garric, 2008; Christensen et al., 2009; Hughes et al., 2013; Jones et al., 2002; Kaplan, 2013; Ortiz de Garcia et al., 2013). However, it seems unlikely that these antibiotics, which have been identified as amongst those of the greatest concern, are actually causing significant acute toxicity problems today for wildlife on their own. But, there may be a case for mixture effects leading to a higher net concern for the antibiotics (Backhaus and Karlsson, 2014). The question of the promulgation of environmental antibiotic resistance was not addressed in this study, but the presence of hundreds of ng/L of some antibiotics in high exposure hotspots in European rivers should be noted.

Conflicts of interest

I certify that there is no conflict of interest with any organisation regarding the material discussed in this manuscript

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Table S1.

Proportion of parent drug excreted by patients for ciproflaxacin (CIP), Sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY)

Reference	CIP excretion (%)	SUF excretion (%)	TRI excretion (%)	ERY excretion (%)
(Schwartz and Rieder, 1970)	NA*[3]	15	48	NA
(Dollery, 1991)*	25-45	30	44-48	2.5
(Vree and Hekster, 1987)	NA	10	NA	NA
(Huschek et al., 2004)	NA	15-20		25
(Straub, 2013)	NA	NA	Up to 60	
(Bryskier et al., 1993)	NA	NA		12-45
(McArdell et al., 2003)	NA	NA		5-10
(Carballa et al., 2008)		15-30	43	4-10
(ter Laak et al., 2010)		20	45	
Expected excretion*[2]	**35**	**18**	**46**	**17**
Worst case excretion	**45**	**30**	**60**	**45**
Best case	**25**	**10**	**43**	**2.5**

*[1]This reference also gives intra venous excretion rates. However, in the UK the IV route for antibiotics is typically less than 1% of the total and so the higher excretion from this route was not considered important.

*[2] Calculated as a weighted mean taking into account the number of patients

*[3] NA Information not presented or available

Table S2.

Proportion of ciproflaxacin (CIP), Sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY) removed in sewage treatment

Reference	Number of STPs	CIP removal (%)	SUF removal (%)	TRI removal (%)	ERY removal (%)
(Roig, 2010)	1	80	NA*[2]	NA	NA
(Giger et al., 2003)	3	81	NA	NA	NA
(Golet et al., 2003)	1	84	NA	NA	NA
(Zorita et al., 2009)	1 (n=5)	90	NA	NA	NA
(Schaar et al., 2010)	1	NA	45	58	48
(Roberts and Thomas, 2006)	1	NA	NA	3	79
(Carballa et al., 2004)	1 (n=2)	NA	60 (55-65)	NA	NA

(Gobel et al., 2005)	2 (n=5)	NA	35		0
(Gobel et al., 2007)	2 (n=5)	NA	14 (0-60)	8 (0-20)	1 (0-6)
(Wahlberg et al., 2011)	3 (n=13)	NA	45	32	NA
(Gardner et al., 2013b)	16	NA	NA	NA	21-42
(Bendz et al., 2005)	1	NA	0	49	NA
(Xu et al., 2007)	2-3	NA	50 (35-65)	NA	35 (15-45)
(Leung et al., 2012)	7	NA	70 (65-75)	NA	20 (5-45)
(Miege et al., 2009)	(n=4-35)	70	59	16	65
(Lindberg et al., 2005)	5 (n=10)	87	31	0	NA
(Straub, 2013)	63	NA	NA	30	NA
(Castiglioni et al., 2006)	6	61	44	NA	0
(Vieno et al., 2007)	12	88	NA	NA	NA
(Batt et al., 2006)	2	NA	9	25 (1-50)	NA
(Ternes et al., 2007)	1	NA	24	69	25
(Xu et al., 2007)	4	NA	24 (0-64)	NA	26
Expected removal*		**76**	**48**	**24**	**36**
Worst case removal		**61**	**0**	**0**	**0**
Best removal		**90**	**75**	**69**	**79**

*Calculated as a weighted mean taking into account the number of STPs in the study

*[2] NA Information not presented or available

Table S3.

Comparing predicted Europe-wide 90%ile values to measured river concentrations (ng/L) for ciproflaxacin (CIP), sulfamethoxazole (SUF), trimethoprim (TRI) and erthromycin (ERY).

Reference	location	River	CIP	SUF	TRI	ERY
GWAVA 90%ile expected case scenario (mean value)*[1]	Europe	all	19	27	45	61
GWAVA 90%ile worst case scenario (mean value)*[1]	Europe	all	30	83	76	216

(Zhou et al., 2009)	England	Sussex Ouse	NA*[2]	6 to 10	NA	NA
(Boxall et al., 2014)	England	Not stated	NA	NA	0.8-50	NA
(Kasprzyk-Hordern et al., 2008)	Wales	Taff & Ely	NA	0 to 4	0 to 183	0 to 351*[3]
(Kasprzyk-Hordern et al., 2007)	Wales	Taff	NA	<0.5	<1.5	0-22*[3]
(Kasprzyk-Hordern et al., 2007)	Poland	Warta	NA	26-60	0 to 27	<0.5
(Banzhaf et al., 2013)	Luxembourg	Mess	NA	3	NA	NA
(Pailler et al., 2009)	Luxembourg	Mess/Alzette	NA	0-22	NA	NA
(Martin et al., 2011)	Spain	Guadalquivir	NA	LOD	LOD	NA
(Garcia-Galan et al., 2011)	Spain	Ebro	NA	30 median	NA	NA
(Gros et al., 2007)	Spain	Ebro	NA	4 to 45	3 to 17	4 to 34
(Fernandez et al., 2010)	Spain	Madrid area	NA	7	12	NA
(Pena et al., 2007)	Portugal	Mondego	80-119	NA	NA	NA
(Madureira et al., 2010)	Portugal	Douro	NA	0 to 53	0 to 16	NA
(Dinh et al., 2011)	France	Seine	NA	4 to 25	0 to 8	0 to 4
(Tamtam et al., 2008)	France	Seine	Below 10	23 to 69	11 to 27	NA
(Nodler et al., 2011)	Germany	Leine	NA	61 median	NA	NA
(Loos et al., 2010)	Hungary	Danube	NA	16 median	NA	NA
(Loos et al., 2009)	Europe	several	NA	15 median	NA	NA
(Houtman et al., 2013)	Holland	Meuse	NA	20 median	4 median	NA

*[1]This is the 90%ile value from the 372 simulations per cell. The mean is derived from the 177,000 cells covering the landmass of Europe

*[2] NA Information not presented or available

*[3] erythromycin-H2O is a metabolite of erythromycin