



Antibiotics in the aquatic environment – A review – Part I

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ABSTRACT

Although antibiotics have been used in large quantities for some decades, until recently the existence of these substances in the environment has received little notice. It is only in recent years that a more complex investigation of antibiotic substances has been undertaken in order to permit an assessment of the environmental risks they may pose. Within the last decade an increasing number of studies covering antibiotic input, occurrence, fate and effects have been published, but there is still a lack of understanding and knowledge about antibiotics in the aquatic environment despite the numerous studies performed. This review addresses the present state of knowledge concerning the input, occurrence, fate and effects of antibiotics in the environment. It brings up important questions that are still open, and addresses some significant issues which must be tackled in the future for a better understanding of the behavior of antibiotics in the environment, as well as the risks associated with their occurrence. Questions related to resistance in the environment that may be caused by antibiotics will be addressed in the second part.

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1. Introduction

1.1. Scope of this article

In contrast to the properties and effects desired from the therapeutic application of antibiotics, these same properties are often disadvantageous for those target and non-target organisms present in the environment. A review of pharmaceuticals, including antibiotics for veterinary use and related environmental issues on a global scale, was recently published by Sarmah et al. (2006). Therefore, this aspect and others, such as the fate of antibiotics in soil or surface water run-off after application of manure (Kreuzig and Hölte, 2005), or in the use of sewage sludge for land amendment is not discussed in detail in this review. A detailed review on analytical methods for the determination of antibiotics in the aquatic environment has been recently published (Hao et al., 2007). Measurements conducted during the last decade showed that the concentrations of antibiotics in municipal sewage, hospital effluents, influents and effluents of STPs, surface water and ground water are mostly in the same range, respectively. Therefore, as for the concentration of antibiotics in the aquatic environment, only some of the recent literature published on the topic is cited in the following sections.

1.2. Some terminology

An antibiotic in a broader sense is a chemotherapeutic agent that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoa. Other terms which are often used are chemotherapeutics or antimicrobials, however, these terms are not synonymous. For example, antimicrobials can also be effective against viruses. The expression “chemotherapeutic” refers to compounds used for the treatment of disease which kill cells, specifically microorganisms or cancer cells. In popular usage, it often refers to anti-neoplastic drugs used to treat cancer. The term “chemotherapeutic” may also refer to antibiotics (“antibacterial chemotherapy”). The term antibiotic originally referred to any agent with biological activity against living organisms; however, “antibiotic” now refers to substances with antibacterial, anti-fungal, or anti-parasitical activity. There are currently about 250 different chemical entities registered for use in medicine and veterinary medicine (Kümmerer and Henninger, 2003). An overview of the most important classes and groups is given in Table 1. Excretion rates and further details on properties and metabolism is found in the medical and pharmaceutical literature as well as on the internet (<http://pubchem.ncbi.nlm.nih.gov/>; Kümmerer and Henninger, 2003; Lorian, 2005).

The first antibiotics were of natural origin, e.g. penicillins produced by fungi in the genus *Penicillium*, or streptomycin from bacteria of the genus *Streptomyces*. Currently, antibiotics are obtained by chemical synthesis, such as the sulfa drugs (e.g. sulfamethoxazole), or by chemical modification of compounds of natural origin. Many antibiotics are relatively small molecules with a molecular weight of less than 1000 Da. The classical definition of an antibiotic is a compound produced by a microorganism which inhibits the growth of another microorganism. Over the years, this definition has been expanded to include synthetic and semi-synthetic products. In this article the term “antibiotic” refers only to drugs that kill or inhibit bacteria, fungi or viruses. Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents

in the treatment of infectious diseases in humans, animals and plants.

1.3. Antibiotics are special

Antibiotics can be grouped by either their chemical structure or mechanism of action. They are a diverse group of chemicals that can be divided into different sub-groups such as β -lactams, quinolones, tetracyclines, macrolides, sulphonamides and others. They are often complex molecules which may possess different functionalities within the same molecule. Therefore, under different pH conditions antibiotics can be neutral, cationic, anionic, or zwitterionic (Fig. 1a and b and Fig. 2a and b). Because of the different functionalities within a single molecule, their physico-chemical and biological properties such as $\log P_{ow}$ (Cunningham, 2008), sorption behavior, photo reactivity and antibiotic activity and toxicity may change with pH.

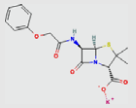
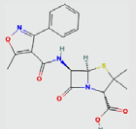
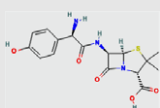
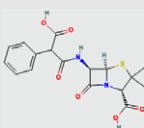
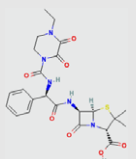
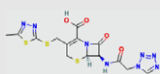
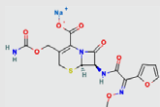
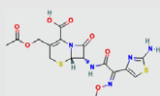
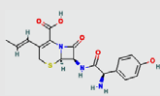
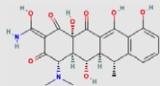
Ciprofloxacin (Fig. 1a), for example, possesses both basic and acidic functionalities. The acid constants are 6.16 and 8.63. At a pH of 7.4, the iso-electric point of ciprofloxacin, the molecule carries both a negative and a positive charge, i.e. it is neutral as an entity despite these charges within the molecule (Fig. 1b). Solubility, hydrophobicity and hydrophilicity, and therefore $\log K_{ow}$ or the distribution coefficient $\log K_D$, are all dependant upon pH. Trivedi and Vasudevan (2007) investigated ciprofloxacin speciation as a function of pH in aqueous solution and in the presence of dissolved ferric ions and goethite. The ciprofloxacin zwitterions appeared to interact via both carboxylate oxygen atoms to form bidentate chelate and bridging bidentate complexes within colloidal iron oxide-ciprofloxacin precipitates, and bidentate chelates on the goethite surface. However, the structure of the aqueous ferric-ciprofloxacin complexes remained unclear. Solution chemistry (pH, ionic strength (I), and sorbate-to-sorbent ratio) effects on ciprofloxacin sorption to hydrous oxides of Al (HAO) and Fe (HFO) were investigated by Gu and Karthikeyan (2005) using macroscopic and spectroscopic analyses. Sorption showed a strong pH-dependent behavior when following the fraction of zwitterionic species over the entire pH range studied. Analysis indicated that different types of ciprofloxacin surface complexes are formed with HAO and HFO, while a monodentate mononuclear complex (with $-\text{COO}^-$) appeared likely to form between ciprofloxacin and HAO, keto O, and one O from COO^- . These seem to be involved in the formation of a six-membered ring with Fe on the HFO surface. Ceftazidime is an inner salt, so one may even expect different chemical species to form depending on pH (Fig. 2a and b). Tetracycline removal by adsorption onto struvite was affected by the pH of the solution, contact time, and struvite concentration (Basakcildan-Kabakci et al., 2007). The lowest tetracycline removal (8.4%) was observed at pH 7.7, the dissociation constant ($\text{p}K_{a(2)}$) of tetracycline. The decomposition of penicillin G was strongly dependent on the pH of the aqueous phase (Aksu and Tunc, 2005).

2. Sources of antibiotics in the environment

2.1. Natural background

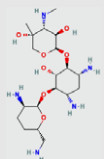
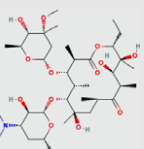
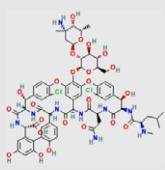
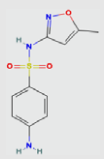
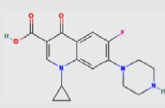
The question of natural background concentrations of antibiotics is important for the risk assessment of antibiotics. Several antibiotics such as some β -lactams, streptomycins, aminoglycosides

Table 1
Important classes and groups of antibiotic compounds.

Class	Group	Subgroup	Example	Chemical structure
β -lactams	Penicillins	Benzyl-penicillins	Phenoxypenicillin	
		Isoxazolylpenicillins	Oxacillin	
		Aminopenicillins	Amoxicillin	
		Carboxypenicillins	Carbenicillin	
		Acylaminopenicillins	Piperacillin	
	Cephalosporins	Cefazolin group	Cefazolin	
		Cefuroxim group	Cefuroxim	
		Cefotaxim group	Cefotaxim	
		Cefalexin group	Cefprozil	
	Carbapenems	-	-	Meropenem
Doxycycline				
Tetracyclines	-	-		

(continued on next page)

Table 1 (continued)

Class	Group	Subgroup	Example	Chemical structure
Aminoglycosides	–	–	Gentamicin 1c	
Macrolides			Erythro-mycin A	
Glycopeptides			Vancomycin	
Sulfonamides			Sulfamethoxazole	
Quinolones			Ciprofloxacin	

and others are produced by soil bacteria. The group of Actinomycetes includes many soil bacteria such as Streptomyces. Streptomyces produce antibiotics. The antibiotic activity from local soil samples is variable and requires the examination of several samples to find a few that produce zones of inhibition. To the authors' best knowledge there have been no findings of tetracyclines in soils which have not been fertilized with manure containing tetracyclines. The concentration has always been below the detection limit in untreated soils used as controls when studying the input and fate of tetracyclines in soils. This situation may be different in tropical soils as the bacteria producing tetracycline occur naturally in higher density in such soils.

Bacterial density is much lower in the free water phase compared to sewage sludge or soil to expect measurable concentrations of antibiotics of natural origin. One may assume that sediments resemble soils, as they are also solid media with both aerobic and anaerobic compartments. In both soils and sediments, bacteria are less mobile than in the free water phase, and the bacterial density is higher. Up to now, there has been no report of the production of antibiotics in sediments or the aquatic environment. A conclusion on this open question cannot be drawn as long as we do not have any results for naturally occurring antibiotics and their concentrations in sediments.

2.2. Production and manufacturing

Emissions from production plants have been thought of as being of minor importance. However, only recently it has been found that

in some Asian countries concentrations of up to several mg L^{-1} could be found in effluents for single compounds (Larsson et al., 2007; Li et al., 2008a,b). In developed countries a manufacturing plant can also make a significant contribution to total antibiotic concentration in the influent of a sewage treatment plant (STP), as has been shown only recently for the VEAS STP in Oslo (Thomas, 2008).

2.3. Usage

Antibiotics are used extensively in human and veterinary medicine, as well as in aquaculture, for the purpose of preventing (prophylaxis) or treating microbial infections. Several hundred different antibiotic and antimycotic substances are used in human and veterinary medicine, e.g. more than 250 in Germany (Kümmerer and Henninger, 2003). Internationally comparable data on antibiotic consumption is scarce, and whatever information is available is heterogeneous. Usage patterns may be different in different countries (Kümmerer, 2008, submitted for publication). In the USA for instance, the use of streptomycin in fruit growing is widespread, whereas its use for this purpose is banned in other countries such as Germany. Wise (2002) estimated antibiotic consumption worldwide to lie between 100,000 and 200,000 ton per annum. In 1996, about 10,200 ton of antibiotics were used in the EU, of which approximately 50% was applied in veterinary medicine and as growth promoters. According to data supplied by the European Federation of Animal Health (European Federation of Animal Health, 2001), in 1999 there were a total of 13,216 ton of

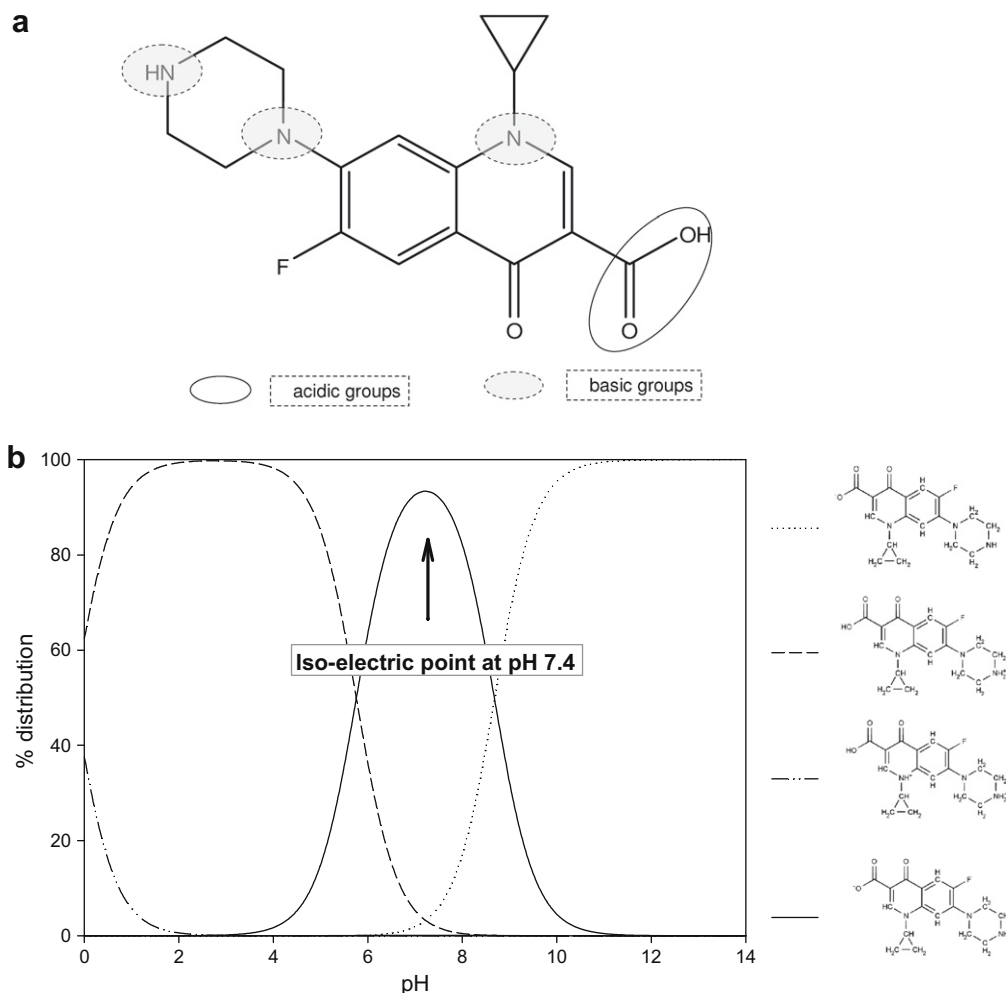


Fig. 1. Chemical structure of ciprofloxacin (a); at different pH ciprofloxacin carries different electrical charges i.e., different chemical species are present (b). (Calculator Plugins were used for structure property prediction and calculation, Marvin 5.0, 2006, ChemAxon (<http://www.chemaxon.com>)).

antibiotics used in the European Union and Switzerland, 65% of which was applied in human medicine. In the United States, one estimate is that 50% of the 22,700 metric tons of all antimicrobials prescribed annually are for humans and 50% for use in animals, agriculture and aquaculture. A more recent report estimated that US livestock producers use approximately 11,200 metric tons of antimicrobials for non-therapeutic purposes primarily to promote the growth of cattle, hogs, and poultry. Clinical uses are estimated at about 10% of total antimicrobial use (Union of Concerned Scientists, 2001).

2.4. Human medicine

Consumption for humans in total, per capita and the individual share of each compound varies from country to country. Antibiotic prescription rates and intake without prescription vary markedly between countries (Mölstad et al., 2002). Varying levels of use of single compounds is quite common. Vancomycin, for example, is heavily used in the USA, whereas in Germany it is only used in cases in which all other possible compounds which have proven to be ineffective due to resistance. Data on the country-specific use for groups of antibiotics in different countries are available from different sources but mostly as DDD.¹ Country-specific con-

sumption for groups of antibiotics in human medicine in DDDs can be found for Europe on the ESAC homepage (<http://www.esac.uu.ac.be/main.aspx?c=ESAC2&n=10661>). Antibiotic use (expressed as DDD per day and capita) ranges from 8.6 to 36 in Europe. The volume of use usually only refers to a nationwide scale, and analytical data do not cover local volumes of use.

It was found that β -lactam antibiotics, including the sub-groups of penicillins, cephalosporins and, as a marginal fraction carbapenems and others, make up the largest share of human use antibiotics in most countries. They account for approximately 50–70% of total antibiotic use. In most countries, sulphonamides, macrolides, and fluoroquinolones follow in decreasing order of use (<http://www.esac.uu.ac.be/main.aspx?c=ESAC2&n=1063>). Cars et al. (2001) obtained data for non-hospital antibiotic sales for 1997 from the 15 member states and analysed these according to the Anatomic Therapeutic Chemical (ATC) classification system, and expressed them as defined daily doses per 1000 people per day. Sales of antibiotics varied more than four-fold: France (36.5), Spain (32.4), Portugal (28.8), and Belgium (26.7) had the highest sales, whereas the Netherlands (8.9), Denmark (11.3), Sweden (13.5), Germany (13.6), and Austria (12.4) had the lowest. There was also great variation in the use of different classes of antibiotics. In another study (Vaccheri et al., 2002) antibiotic consumption was 16.5 DDD/1000 inhabitants/day (DDD per 1000 inhabitants and day) in Ravenna (Italy) and 10.4 DDD/1000 inhabitants/day in Funen (Denmark). Italian children received a greater amount (four-fold in DDDs) of

¹ Defined daily dose according to WHO.

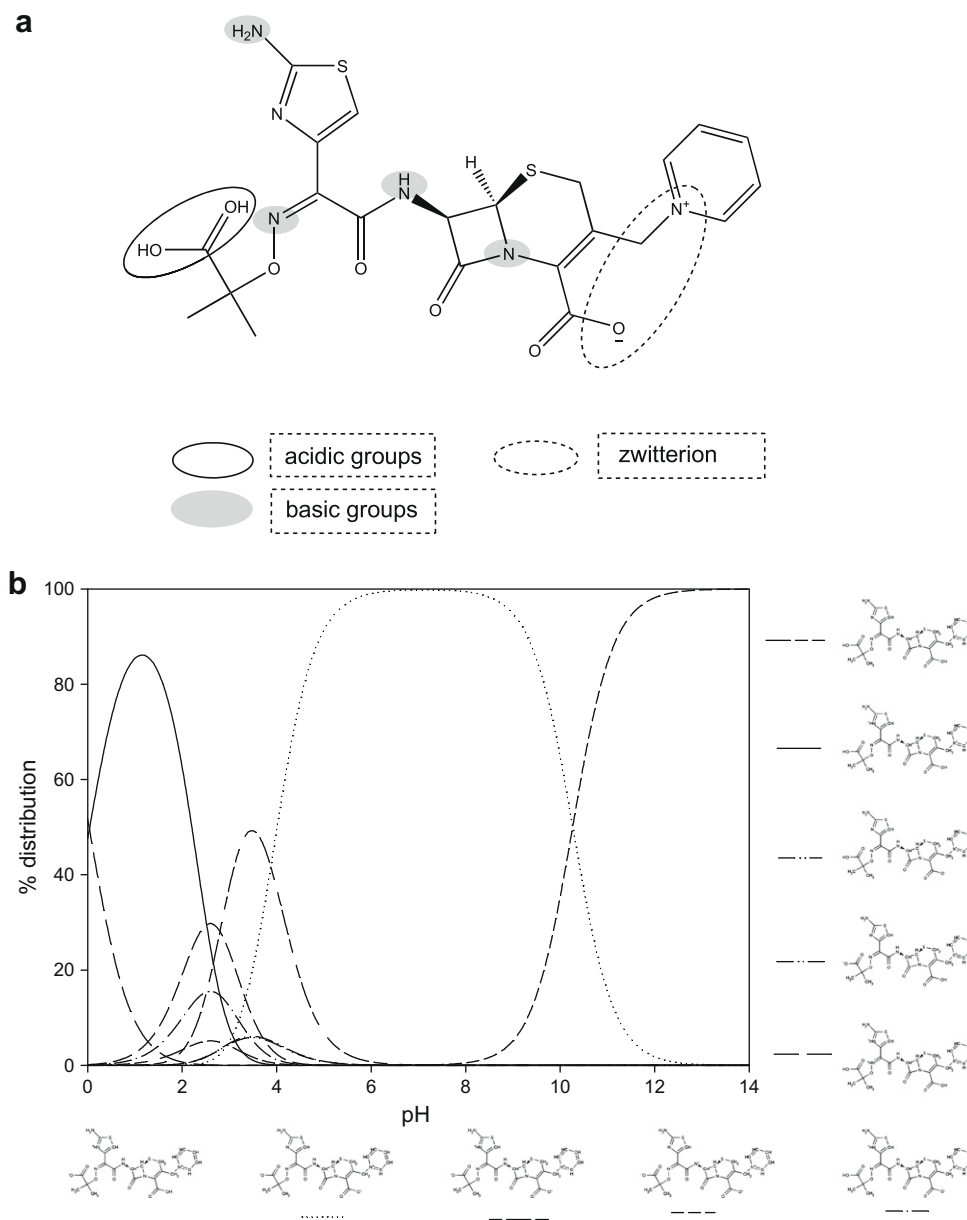


Fig. 2. Chemical structure of ceftazidime (a); ceftazidime forms an internal zwitterion and can form additional chemical species as a function of pH (b). (Calculator Plugins were used for structure property prediction and calculation, Marvin 4.1.1, 2006, ChemAxon (<http://www.chemaxon.com>)).

antibiotics than Danish ones. In Italy, injectable antibiotics (third generation cephalosporins or aminoglycosides) accounted for 4% of total DDDs and 11% of exposed subjects. In Funen, use of injectable antibiotics was negligible. The bulk of prescription (90% of total DDDs) was made up of eight (out of 38) different antibiotics in Denmark, mainly narrow-spectrum penicillins and macrolides (1st: phenoxymethylpenicillin), and of 18 (out of 74) antibiotics in Italy, mainly broad-spectrum penicillins, macrolides, fluoroquinolones and cephalosporins.

Regional and local consumption also may be different within a single country: 9.6–17.3 DDDs per capita and day in Germany (de Wirth et al., 2004). For the EU, in total 22 g per capita and year would be due to medical use. For the USA it is estimated to be approximately 17 g per capita and year, when calculated from the available data for use in human medicine (Kümmerer, 2004). However, these data include some uncertainty. In Germany nationwide average use of antibiotics in 1998 was 4.95 g per capita and year, whereas it was 2.9 g per capita and year in a small town

(9000 inhabitants, no hospital present but 2 elderly peoples' homes). If the share of the average use in hospitals in Germany is added, the result would be 3.85 g per capita and year. These data demonstrate that there should be a potential for a reduction in antibiotic use without negative health consequences.

If antibiotics are sold over the counter (oxytetracycline), i.e. without any prescription, consumption could be still higher. According to different legislation and differing degrees of importance ascribed to the use of antibiotics, reliable data providing information on the total use and the patterns of antibiotic use and per capita consumption exist for only a few countries. Excretion rates for the unchanged active compound cover a broad range (10–90%, ceftazidime for example less than 10%). On average, if the volume for all antibiotics used is totalled the metabolic rate is estimated to be 30% (Kümmerer and Henninger, 2003), i.e. 70% of the used APIs are excreted unchanged into waste water. The data presented above show that at least at the level of specific compounds general data on the consumption of antibiotics may be misleading.

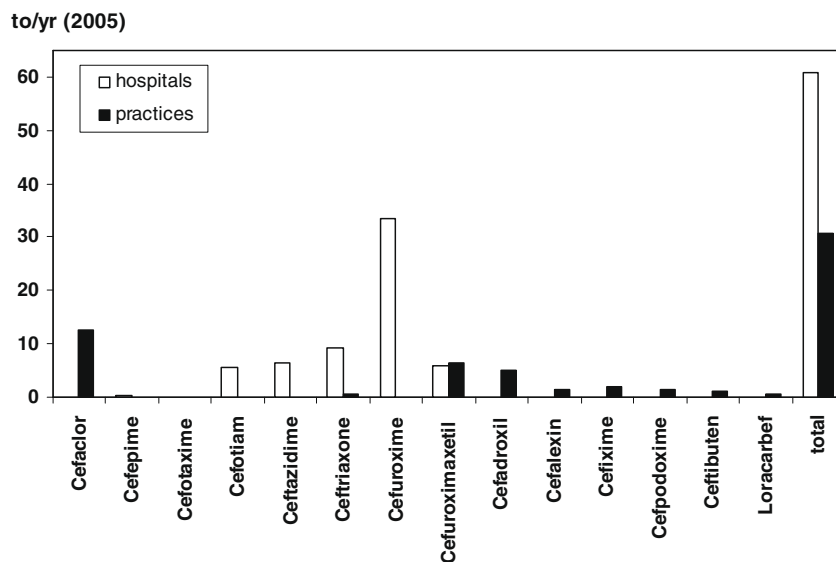


Fig. 3. Use of cephalosporins (cephalosporins, ATC-Code J01DB-E) in German hospitals with maximum service spectrum. Projections, calculated from: hospitals: University Medical Centre Freiburg via hospital bed proportion; practices: Arzneverordnungen – Report 2006: statutory health insurance – proprietary medicinal (Kümmerer, 2008).

Evaluation on a case-by-case basis may be necessary to assess substance flows of antibiotics.

In contrast to general expectation, hospitals are not the main source of pharmaceuticals in municipal sewage (Kümmerer, 2008; Schuster et al., 2008). De Wirth et al. (2004) found that on the basis of DDDs, the use of antibiotics in hospitals accounts for 5–20% of the total antibiotic use in European hospitals. Community use is reported to be 70% in the UK (House of Lords, 1998) and 75% in the US (Wise, 2002). For a local STP in Oslo it was found that less than 10% of certain analysed antibiotics were from hospitals (Thomas et al., 2007). In Germany, about 75% of antimicrobials are used in the community and 25% in hospitals (Kümmerer and Henninger, 2003). In total, 412 ton of antibiotics were used in Germany 1998. Taking the compound specific metabolization rates into account, 305 ton are emitted into waste water, of which 92 ton were due to hospitals (Kümmerer and Henninger, 2003). Hospitals are the major source of antibiotics for only the cephalosporin group of antibiotics (Fig. 3). However, these roughly calculated data are based on data from hospitals offering a maximum service spectrum. In these hospitals 3rd and 4th generation antibiotics, of which cephalosporins make up a large share, are overrepresented. The data demonstrate that there are no point sources but diffuse input by the general public/STPs.

As for the metabolism of active compounds in humans there is a wide range in the degree to which these compounds are metabolized (Lorian, 2005; Kümmerer and Henninger, 2003). Some compounds are metabolized by 90% or more, while others are metabolized by only 10% or even less. However, when the amounts used for each active compound is multiplied by its excretion rate, then even some compounds with a high metabolization rate are most important.

Based on such data and nationwide consumption rates it was found that 70% of the total amount used in Germany, for example is excreted unchanged, i.e. as still-active compounds. Metabolism takes place most often in the liver. Often the metabolites are more water soluble than the parent compounds, leading to their excretion with urine. In some cases metabolism results in a of chloramphenicol. However, sometimes the formation of metabolites can result in compounds which are more toxic to humans than the parent compound. An example of this can be seen in the acetylation of sulfamethoxazole. However, the importance of cleaving back the acetyl derivative to sulfamethoxazole under environmental condi-

tions has been reported (Göbel et al., 2005). For some β -lactams cleavage of the β -lactam ring has been shown (Längin et al., 2009; Helland et al., submitted for publication). However, knowledge about these issues is scarce.

2.5. Animals

Because international data are based only on estimates, the true volume of antimicrobial use in the agri-food sector is not known. Estimates of the amounts of antimicrobials used are subject to confusion (see for example data from the USA (http://www.hc-sc.gc.ca/dhp-mpps/pubs/vet/amr-ram_issue-enjeux_e.html); for most countries no data or only rough estimates are available (see below). Consumption by animals for the purposes of prevention or therapy is largely determined by modern animal breeding and fattening methods and conditions. Antibiotics are also used to promote the growth of animals (Gaskins et al., 2002) in some countries where they are used at low doses in animal feeds and are considered to improve the quality of the product, with a lower percentage of fat and higher protein content in the meat (Cromwell, 2002). The use of even small amounts of antibiotics is associated with the selection of resistance (see below) in pathogenic bacteria. It has been argued that the use of antibiotic growth promoters imposes a selective pressure for bacteria that are resistant to antibiotics that may be used in clinical or veterinary practice, thus compromising the continued use of antimicrobial chemotherapy. Alternatives to improve the quality of the product, with a lower percentage of fat and a higher protein content in the meat without using antibiotics are available (http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/555_EN.HTM). In the European Union and some other countries such as Sweden and Switzerland, the use of antibiotics as growth promoters in animal farming has been banned for the last several years.

Some compounds may be used for purposes other than human or veterinary medicine: Antibiotics such as streptomycins are used in fruit growing, while others are used in bee-keeping.

2.6. Plant agriculture

Antibiotics have been used since the 1950s to control certain bacterial diseases of high-value fruit, vegetable, and ornamental

plants. Today, the antibiotic most commonly used on plants is streptomycin with oxytetracycline to a minor extent. Primary uses are on apple, pear, and related ornamental trees for the control of fire blight caused by *Erwinia amylovora*. In the USA, antibiotics applied to plants account for less than 0.5% of total antibiotic use (McManus et al., 2002). Most of the antimicrobials, consisting mainly of streptomycin and oxytetracycline, are used in controlling bacterial diseases of tree fruits. 13.835 metric tons of streptomycin was applied in the USA (<http://www.apsnet.org/online/feature/Antibiotics/>). To be a viable candidate for disease control, the antibiotic needed to: (i) be active on or inside of the plant; (ii) tolerate oxidation, UV irradiation, rainfall, and high temperatures. These properties are exactly the ones causing problems in the environment. However, data on streptomycin concentrations in soil for growing fruit are missing. Again, due to different regulations the situation is different in different countries. In Germany for example the use of streptomycin in fruit growing needs a special allowance which is not generally available except for on a case-by-case basis.

2.7. Aquaculture

The current definition of aquaculture, according to FAO, is “the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants”. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. Antibiotics authorized for use in aquaculture are oxytetracycline, florfenicol, premix, sarafloxacin, erythromycin sulphenamides potentiated with trimethoprim or ormethoprim (Serrano, 2005). The usage of antimicrobial drugs in farmed fish in Norwegian aquaculture for the period 2000–2005 was investigated by Grave et al. (2008). An increase in the usage of antibiotics in Norwegian aquaculture was observed from 2002 to 2005, which was accounted for by newly-farmed fish species (other than Atlantic salmon and rainbow trout), especially Atlantic cod. However, such specific data are missing for most countries. The contribution of the aquaculture sector is not expected to be a significant percentage of the non-human use of antimicrobials in the USA (http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_issue-enjeux_e.html). The situation in many other countries is not clear.

3. Occurrence

Antibiotics can be more or less extensively metabolized by humans and animals. After administration, antibiotics for human use or their metabolites are excreted into the effluent and reach the sewage treatment plant (STP). The non-metabolized fraction is excreted as a still-active compound. Looking at all compounds, approx. 70% of the consumed amount of antibiotics in Germany is excreted unchanged (Kümmerer and Henninger, 2003). Antibiotics are only partially eliminated in sewage treatment plants. If they are not eliminated during the purification process, they pass through the sewage system and may end up in the environment, mainly in the water compartment. Residual amounts can reach surface waters, groundwater or sediments.

Active substances discharged with liquid manure can be washed off from the top soil after rain. Furthermore, direct discharge, especially from poultry processing, meat processing, and aquaculture, as well as from pets (e.g. aquariums) is also possible and can contribute towards an increase in the total concentration of antibiotics in sewage and surface water.

3.1. Waste water, surface water, ground water, drinking water, and seawater

Research has quite extensively studied the presence of antibiotics in the environment (for a short overview see Table 2; Alexy and Kümmerer, 2006). As for other pharmaceuticals, it has been found that the concentrations of antibiotics measured in different countries are in the same range of concentrations in the different compartments such as sewage and surface water, respectively (Batt and Aga, 2005; Botitsi et al., 2007; Hernández et al., 2007; Chang et al., 2008; Peng et al., 2008; Duong et al., 2008; Martins et al., 2008). In general, concentrations were in the higher $\mu\text{g-per-litre}$ range in hospital effluent, in the lower $\mu\text{g-per-litre}$ range in municipal waste water, and in the higher and lower $\mu\text{g-per-litre}$ range in different surface waters, ground water and sea water in a harbour (Xu et al., 2007) – if found at all in the latter. Losses of sulphonamide antibiotics from grassland to a brook after application of manure were strongly influenced by the weather conditions (Stoob et al., 2007). The compounds that have been analyzed up to now are from a number of different important classes of antibiotics. They include primarily macrolides (e.g. clarithromycin, erythromycin, roxithromycin), aminoglycosides (which include for example amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, and of which only gentamicin has been investigated), tetracyclines (tetracycline, chlortetracycline, oxytetracycline, demeclocycline (not analyzed) doxycycline), sulphonamides (many compounds among them sulphadimethoxine (not investigated), sulphanilamides (only a few investigated), sulfamathoxazole, sulphasalazine) and quinolones (1st generation: nalidixic acid (rarely investigated); 2nd generation: e.g. ciprofloxacin, lomefloxacin (not investigated), norfloxacin, ofloxacin; 3rd generation: e.g. levofloxacin, sparfloxacin (not investigated), tosufloxacin (not investigated); 4th generation (not investigated): e.g. clinafloxacin, gemifloxacin, moxifloxacin, sitafloxacin) to name just a few. Quinolones (ciprofloxacin most often analysed) and other pharmaceuticals have been detected in the effluents of hospitals (Hartmann et al., 1998; Lindberg et al., 2004; Turiel et al., 2005a; Brown et al., 2006; Thomas et al., 2007; Duong et al., 2008; Martins et al., 2008) up to a low $\mu\text{g-per-litre}$ range.

The occurrence of β -lactams (including penicillins, cephalosporins carbapenems, monobactams, β -lactamase inhibitors), has not been covered frequently, despite the fact that β -lactams account for by far the highest proportion of consumption (Färber, 2002; Christian et al., 2003). It is not clear whether they are not present in the aquatic environment because of the possible cleavage of the β -lactam ring, whether this finding is due to the fact that they have not been analysed, or whether it is due to possible analytical shortcomings and difficulties. In one study β -lactams were detected in the lower $\mu\text{g-per-litre}$ range in hospital effluent and in the influent of a municipal STP (Christian et al., 2003). The concentrations found for β -lactams are low compared to the ones expected from the extensive use of β -lactams (some $\mu\text{g L}^{-1}$ or less found instead 20–30 $\mu\text{g L}^{-1}$ expected). Antibiotics have also rarely been found in drinking water (Ye et al., 2007).

3.2. Sewage sludge and sediments

Human and veterinary antibiotics are present in sediments. Kim and Carlson (2007) detected tetracyclines, sulphonamides and macrolides. The sediment concentration measured in an agriculture-influenced river was much higher than in the overlying water matrix, indicating the input of antibiotics with surface run-off from agricultural fields. In intensive fish farming, infections are treated by feeding antimicrobial agents directly into the water. The substances used in fish farming can enter the sediments directly from the water without undergoing any kind of

Table 2

Examples of measured concentrations of antibiotics in the aquatic environment.

Antibiotic	Sewage treatment plant effluent	Surface water	Ground water ^a /bank filtrate	References
ng L ⁻¹				
<i>Penicillins</i>				
Penicillin	up to 200	up to 3		Färber (2002)
Flucloxacillin		7		Christian et al. (2003)
Piperacillin		48		Christian et al. (2003)
<i>Makrolides</i>				
Makrolide				
Azithromycin	up to 700	up to 20	up to 2 [*]	Färber (2002)
Erythromycin-H ₂ O	up to 287	up to 3		Christian et al. (2003)
	up to 6000	up to 1700	up to 49	Giger et al. (2003a,b)
		up to 190		Sacher et al. (2002)
		up to 1700		Hirsch et al. (1999)
		up to 15.9		Christian et al. (2003)
Clarithromycin	up to 400			Kolpin et al. (2002)
	up to 328	up to 65		Calamari et al. (2003)
	up to 240	up to 260		Alexy et al. (2006)
		up to 37		Giger et al. (2003a,b)
		up to 20.3		Hirsch et al. (1999)
Roxithromycin	up to 38			Christian et al. (2003)]
	up to 68			Calamari et al. (2003)
	up to 72			Alexy et al. (2006)
	up to 1000		up to 26	Alexy et al. (2006)
		up to 560		Giger et al. (2003a,b)
		up to 14		Sacher et al. (2002)
		up to 180		Hirsch et al. (1999)
				Christian et al. (2003)
				Kolpin et al. (2002)
<i>Chinolones</i>				
Fluorchinolone				
Fluorchinolone (ciprofloxacin, norfloxacin)	up to 100	up to 5		Färber (2002)
Ciprofloxacin	up to 106	up to 19		Giger et al. (2003a,b)
		9		Christian et al. (2003)
		up to 30		Kolpin et al. (2002)
		up to 26.2		Calamari et al. (2003)
Norfloxacin		up to 120		Kolpin et al. (2002)
Ofloxacin	up to 82			Alexy et al. (2006)
		20		Christian et al. (2003)
<i>Sulfonamides</i>				
Sulfonamide				
Sulfa-methoxazol	up to 1000	up to 40	up to 20 [*]	Färber (2002)
	up to 370			Alexy et al. (2006)
	up to 2000	up to 163	up to 410	Sacher et al. (2002)
		up to 480	up to 470	Hirsch et al. (1999)
		up to 52		Christian et al. (2003)
		up to 1900		Kolpin et al. (2002)
Sulfamethazin			up to 160	Hirsch et al. (1999)
		up to 220		Kolpin et al. (2002)
Sulfamethizol		up to 130		Kolpin et al. (2002)
Sulfadiazin			up to 17	Sacher et al. (2002)
Sulfadimidin			up to 23	Sacher et al. (2002)
		up to 7		Christian et al. (2003)
Sulfadimethoxin		up to 60		Kolpin et al. (2002)
<i>Tetracyclines</i>				
Tetracycline (no more specified)				
Tetracyclin	up to 20	up to 1		Färber (2002)
Chlortetracyclin		up to 110		Kolpin et al. (2002)
Oxytetracyclin		up to 690		Kolpin et al. (2002)
		up to 340		Kolpin et al. (2002)
		up to 19.2		Calamari et al. (2003)
<i>Others</i>				
Trimethoprim				
	up to 38			Alexy et al. (2006)
	up to 660	up to 24		Sacher et al. (2002)
		up to 200		Hirsch et al. (1999)
		up to 12		Christian et al. (2003)
		up to 710		Kolpin et al. (2002)
Ronidazol			up to 10	Sacher et al. (2002)
Chloramphenicol	up to 68			Alexy et al. (2006)
	up to 560	up to 60		Hirsch et al. (1999)
Clindamycin	up to 110			Alexy et al. (2006)
		up to 24		Christian et al. (2003)
Lincomycin		up to 730		Kolpin et al. (2002)
		up to 248.9		Calamari et al. (2003)
Spiramicin		up to 74.2		Calamari et al. (2003)
Oleandomycin		up to 2.8		Calamari et al. (2003)
Tylosin		up to 280		Kolpin et al. (2002)
		up to 2.8		Calamari et al. (2003)

^a Directly impacted by surface water.

purification process. This results in high local concentrations in the water compartment and in the adjoining sediments. This phenomenon had already been investigated more than 20 years ago, where results had demonstrated the presence of antibiotics applied extensively in fish farming in sediments beneath fish farms (Jacobsen and Berglind, 1988; Björklund et al., 1991; Coyne et al., 1994; Migliore et al., 1995). Very little additional information has been published since then as the problem has been well documented.

3.3. Uptake by plants

Several studies have investigated the potential for a range of veterinary medicines to be taken up from soil by plants, and have assessed the potential significance of this exposure route in terms of human health. Soil analyses indicated that, for selected substances, measurable residues of these are likely to occur in soils for at least some months following the application of manure containing these compounds. Some antibiotics are taken up by vegetables such as carrot roots (tubers), lettuce leaves (Boxall et al., 2006) and corn (Kumar et al., 2005; Grote et al., 2007). The test crops corn (*Zea mays* L.), green onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L., Capitata group) absorbed chlortetracycline but not tylosin. (Kumar et al., 2005). The concentrations of chlortetracycline and sulfamethazine in plant tissues were small (2–17 $\mu\text{g kg}^{-1}$ fresh weight), but these concentrations increased with increasing amounts of antibiotics present in the manure. Sulfamethazine was taken up by crops, with concentrations in plant tissue ranging from 0.1 to 1.2 mg kg^{-1} dry weight (Dolliver et al., 2007). The highest plant tissue concentrations were found in corn and lettuce, followed by potato. Experimental studies on the uptake of veterinary medicines into carrot roots (tubers) and lettuce leaves showed that only florfenicol, levamisole, and trimethoprim were taken up by lettuce, whereas diazinon, enrofloxacin, florfenicol, and trimethoprim were detected in carrot roots (Boxall et al., 2006). However, the total accumulation of sulfamethazine in plant tissue after 45 d of growth was less than 0.1% of the amount applied to soil in manure. Results indicate that there is little evidence of an appreciable risk. This exposure route may, however, be important when veterinary medicines have a very low acceptable daily intake (ADI) values where they may elicit subtle effects over prolonged periods, or when exposure is occurring via a number of routes at once (Boxall et al., 2006).

4. Elimination

Elimination only means that the (parent) compound of interest is not detectable anymore by compound specific analysis in the compartment or phase of sampling. It has been removed from the compartment of interest e.g. the water phase. Therefore, removal is an adequate expression for this situation too. The elimination of only the parent compound is also called primary elimination. Primary elimination is normally reported if specific analytical methods such as LC–MS are applied in fate studies. Sum parameters such as DOC loss give a measure of the degree of total elimination. If the compound is fully converted into inorganic salts, full mineralization took place. Only the measurement of carbon dioxide production can give a measure of the degree of mineralization that results in the complete breakdown of a molecule, its metabolites, and transformation products into carbon dioxide, water, and inorganic salts such as sulphate, phosphate, ammonium and nitrate. Since it cannot be excluded that antimicrobials in the environment may have a severe impact on aquatic and terrestrial ecosystems, knowledge about their elimination is of predominant importance.

Elimination of organic compounds in the environment is the result of different processes. These processes can be biotic ones, i.e. biodegradation by bacteria and fungi. Non-biotic elimination processes are sorption, hydrolysis, photolysis, oxidation and reduction. It has to be noted that the results of bio- or photo-degradation studies depend on conditions such as temperature, composition of matrix, latitude etc.

4.1. Sorption

In order to assess the quality of antibiotic data on sorption it is necessary to consider their physical chemical properties. Tolls (2001) reviewed the sorption behavior of antibiotics in soil. Some of this information might be helpful in judging the sorption of antibiotics onto sewage sludge and sediments. However, the content of mineral material is lower in sediments, and aerobic and anaerobic conditions can dramatically differ within a few centimetres. As for sewage sludge, the mineral content is very low. Compared to sediments the lipid concentration is much higher, therefore, more nonpolar, less polar and cationic material is present.

The compilation of sorption coefficients to soil solids (K_d , K_{solid}) by Tolls (2001) demonstrates that these antibiotics display a wide range of mobility ($0.2 < K_d K_{\text{solid}} < 6000 \text{ L kg}^{-1}$). Partition coefficients for the association of tetracycline and quinolone carboxylic acid to dissolved organic matter (K_d , K_{DOM}) vary between 100 and 50000 L kg^{-1} . There are only a few instances in which similar data has been gathered for sediments and sludge. For most of the compounds, the variation is not considerably lower for the organic carbon-normalized sorption coefficient K_{oc} . In addition, the estimation of $\log K_{\text{oc}}$ by $\log K_{\text{ow}}$ leads to significant underestimation of $\log K_{\text{oc}}$ and $\log K_d$, K_D values. This suggests that mechanisms other than hydrophobic partitioning play a significant role in the sorption of antibiotics in soils. However, this share on the sorption may be different in sewage sludge compared to sediment. A number of hydrophobicity-independent mechanisms due to electrical charges such as hydrogen bonding and ionic interactions may be of lesser importance in sediments and sludge. However, this could be pH dependent (see above). This demonstrates that the sorption behavior of antibiotics can be very complex and difficult to assess. It may also be misleading to apply data that have been collected from experiments with a certain matrix such as soils to another one, e.g. sewage sludge. Sorption coefficients, expressed as $\log K_d$, were calculated from fits using the Freundlich isothermic model (Córdova-Kreylos and Scow, 2007). Ciprofloxacin strongly sorbed to all sediments and had $\log K_d$ values, ranging from 2.9 to 4.3. Clay content correlated positively ($r^2 = 0.98$) and pH negatively ($r^2 = 0.99$) to K_d values. The magnitude of the effect of ciprofloxacin on microbial communities inversely correlated with the degree of sorption to the sediments.

Binding to particles or the formation of complexes may cause a loss in detectability, as well as a loss in antibacterial activity. The loss of antibacterial activity, for example, was demonstrated for an aquaculture antimicrobial in seawater driven by the formation of complexes with the magnesium and calcium naturally present in marine water. Tetracyclines are able to form complexes with double cations, such as calcium or magnesium (Christian et al., 2003). Humic substances may alter the surface properties and sites available for sorption and reactions. They can either suppress or promote sorption of organic compounds to mineral surfaces. Limited evidence in the literature points to the reduction in sorption of the antibiotic tetracycline to clay minerals in the presence of humic substances. Gu and Karthikeyan (2008) report strong interaction between humic acids, hydrous Al oxide and the effect of this association on tetracycline sorption. Ligand exchange was proposed as the major mechanism of interaction. Higher organic content resulted in increased nonlinearity of sorption behavior. The

authors conclude that the presence of humic substances, in both dissolved and mineral-bound forms, is likely to increase the environmental mobility of tetracycline compounds. Association of the cationic macrolide clarithromycin to humic acids was largely reversible (Sibley and Pedersen, 2008).

For the sorption of ciprofloxacin to inorganic materials see above (Trivedi and Vasudevan, 2007; Gu and Karthikeyan, 2008). Antibiotics applied in human medicine (e.g. FQs, macrolides) can reach the terrestrial environment through sewage sludge. Golet et al. (2002) Giger et al. (2003a) have confirmed the hypothesis that fluoroquinolones (FQs) become highly enriched in sewage sludge (concentrations ranging from 1.4 to 2.42 mg kg⁻¹ of dry matter). The authors also demonstrated the persistence of FQs in sludge-treated soils several months after application. These results indicate the importance of sludge management strategies to determine whether human-excreted antibiotics enter the environment. FQs have also been found to adsorb onto sediments (Hektoen et al., 1995). Córdova-Kreylos and Scow (2007) measured sorption of ciprofloxacin in sediment samples from three California salt marshes. Sediments were exposed to a ciprofloxacin concentration gradient (0–200 mg L⁻¹ ciprofloxacin). The correlation of K_d values was positive with clay content ($r^2 = 0.98$) and negative with pH ($r^2 = 0.99$).

In the case of sulfadiazine and other sulphonamides it has been found that elimination by sorption to soil particles is a significant process (Tolls, 2001; Kreuzig and Höltge, 2005; Heise et al., 2006; Schmidt et al., 2008). Adsorption studies for penicillin G removal were performed (Aksu and Tunc, 2005). Maximum sorption was observed at an initial pH value of 6.0 and at 35 °C, and the equilibrium uptake by the sorbent increased with increasing initial penicillin G concentration up to 1000 mg L⁻¹. Penicillin G uptake capacity was determined as 330.0 mg g⁻¹ for activated sludge and 375.0 mg g⁻¹ for activated carbon at these conditions (Aksu and Tunc, 2005). However, knowledge about the interaction of antibiotics with sludge and of sediments with sludge in activated sludge plants as well as the subsequent potential for their release back into the environment is still too sparse.

4.2. Photolysis

If a substance is light sensitive, photo-decomposition may be of major significance in the elimination process. In general, data on the sensitivity of antibiotics against light, moisture and temperature can be found in the medical and pharmaceutical literature. Data from the drug registration procedure may give guidance on compounds where photo-decomposition can be expected to play a role. Photo-decomposition takes place mainly in clear surface water. Photochemical decomposition can play an important role in surface water as an additional elimination pathway or for effluent treatment (Viola et al., 2004; Edhlund et al., 2006; Paul et al., 2007; Werner et al., 2007; Hu and Coats, 2007; Hu et al., 2008; Lorenzo et al., 2008). The effectiveness of the process depends on light intensity and frequency. Photo-decomposition may not occur when the compounds are present in turbid water, if the creek, river, or lake is shadowed by trees, or if the compounds are in soil, sewage and sewage pipes since they have low light exposure. Frequency relates to the absorption spectrum of a compound, and the absorption spectrum may be effected by sorption and complexation. Therefore, the effectiveness of photo-transformation in the environment cannot always be derived in a straightforward way from results obtained in laboratory tests. It can also vary with season and the latitude. The effectiveness of depletion processes differ under environmental conditions such as pH or water hardness (Werner et al., 2006) and depends on the type of matrix, location, season and latitude (Kallenborn et al., 2008). It should be noted that incomplete photo-transformation and photo-degradation can lead to more or less stable or more or less toxic compounds

although this does not necessarily have to happen (Cokgor et al., 2006; Arslan-Alaton and Caglayan, 2006; González et al., 2007; Iskender et al., 2007; Paul et al., 2007).

Some antibiotics are light sensitive (e.g. quinolones, tetracyclines, sulphonamides, tylosin, nitrofurantoin antibiotics). However, not all compounds are photo-degradable (Turiel et al., 2005b). The significance and extent of direct and indirect photolysis of antibiotics in the aquatic environment are different for each compound. Studies taking into account indirect photolysis and interaction with dissolved organic matter (DOM) such as humic and fulvic acids are rare (Sukul et al., 2008). Such data would be helpful in order to better understand the fate of antibiotics in surface waters.

Tetracyclines are susceptible to photo-degradation. For example, Samuelsen (1989), investigated the sensitivity of oxytetracycline towards light in seawater as well as in sediments. The antibacterial substance proved to be stable in sediments rather than in seawater. As no mechanism of decomposition other than photo-degradation is known for this antimicrobial (Oka et al., 1989), the substance remains in the sediment for a long period, as shown by Lunestad and Goksøyr (1990). Werner et al. (2006) studied the impact of water hardness (calcium concentration, magnesium concentration, and pH) as a photochemical parameter on tetracycline photolysis. They found that the pseudo-first-order rate constant for tetracycline photolysis at varied Mg²⁺ and Ca²⁺ concentrations relevant to natural conditions can vary by up to an order of magnitude.

Fluoroquinolones are insensitive to hydrolysis and increased temperatures but are degraded by UV light (Burhenne et al., 1997a,b; Thiele-Bruhn, 2003; Viola et al., 2004; Turiel et al., 2005b; Paul et al., 2007; Lorenzo et al., 2008; Vasconcelos et al., submitted for publication).

Sulphanilic acid was found as a degradation product common to most of the sulpha drugs (Boree et al., 2004). In the study of Boree et al., photo-degradation of these drugs in natural water samples (e.g. Lake Superior) was attributed solely to direct photolysis. Rates of UVA-TiO₂ photo-catalyzed sulphamethoxazole degradation are dependent upon several variables, including initial sulphamethoxazole concentration, catalyst phase identity and concentration, electron acceptor identity and concentration, and the presence of non-target water constituents. In contrast, reaction rates are not sensitive to changes in sulphonamide structure (Hu et al., 2008).

The photolysis of tylosin and its photo deactivation in surface water has been described (Hu and Coats, 2007; Werner et al., 2007), as well as for nitrofurantoin antibiotics (Edhlund et al., 2006). Oxolinic acid in water from an eel pond decayed faster under light than in the dark, with a mean half-life of 298 d and 509 d, respectively. High concentration of added oxolinic acid resulted in faster transformation. No difference in oxolinic acid transformation was found between aerobic and anaerobic incubation (Lai et al., 2008).

4.3. Hydrolysis and thermolysis

Another important pathway for the non-biotic elimination of organic substances in the environment is hydrolysis. Some instability in water could be demonstrated for some tetracyclines (Halling-Sørensen, 2000). In general, the hydrolysis rates for oxytetracycline increase as the pH deviates from pH 7 and as temperature increases. The half-lives of oxytetracycline under investigation varied due to differences in temperature, light intensity and flow rate from one test tank to another. However sulphonamides and quinolones are resistant to hydrolysis.

In laboratory biodegradability testing with sewage sludge it has been found that β -lactams are rapidly hydrolysed. This leads to the deactivation of antibiotic activity (Längin et al., 2009). A subsequent step is decarboxylation. Even if the compounds are structurally closely related, the degree of hydrolysis and decarboxylation,

the share of microbial activity in these processes, and their kinetics all differ (Långin et al., 2009). This means that many of the most frequently applied penicillins can probably not be detected in the environment at the expected concentration level. The β -lactam ring of β -lactam antibiotics, e.g. penicillins, can be opened by β -lactamase, an enzyme present in bacteria. Li et al. (2008b) reported thermal decomposition of penicillin G as treatment of the effluent of a production plant. Pouliquen et al. (1992) studied the elimination of an antibiotic in seawater.

4.4. Technical oxidation processes

Antibiotic formulation effluents are well known for the difficulty of their elimination by traditional bio-treatment methods and their important contribution to environmental pollution is due to their fluctuating and recalcitrant nature. For advanced effluent treatment oxidation processes are usually applied. However, ozonation will not work well for all types of molecules. The presence of carbon-carbon double bonds, aromatic bonds or nitrogen is a necessary prerequisite. However, the presence of these structural elements does not guarantee the fast and full degradation or even the mineralization of a molecule.

The effect of ozonation on the degradation of oxytetracycline in aqueous solution at different pH values (3, 7 and 11) was investigated by Li et al. (2008c). The results demonstrate that ozonation as a partial step in a combined treatment concept is a potential technique for biodegradability enhancement for effluents from pharmaceutical industries containing high concentrations of oxytetracycline, provided that the appropriate ozonation period is selected. It has been shown that COD (chemical oxygen demand) removal rates increase with increasing pH as a consequence of enhanced ozone decomposition rates at elevated pH values. The results of bioluminescence data indicate that the initial by-products after partial ozonation (5–30 min) of oxytetracycline were more toxic than the parent compound (Li et al., 2008c).

Sulfamethoxazole was efficiently degraded by ozonation (Dantas et al., 2007). A biodegradability enhancement expressed as the increment of BOD₅/COD (biological oxygen demand after 5 d/chemical oxygen demand) ratio from 0 to 0.28 was observed by the authors after 60 min of ozonation. The acute toxicity of the intermediates was followed by the Microtox test and the toxicity profile showed a slight acute toxicity increment in the first stage of ozonation. pH variation played an important role in TOC and COD removal, promoting their growth with the increment of alkalinity. The complete sulfamethoxazole removal was achieved for an in photo-Fenton process (González et al., 2007). Biodegradability (BOD₅/COD ratio) rose from zero to values higher than 0.3 (treated solutions) which is still quite low. Toxicity and inhibition tests pointed in the same direction: oxidized intermediates showed no toxicity effects on pure bacteria and no inhibition on activated sludge activity. It was found that degradation products formed by ozonation of sulfamethoxazole have effects on mammalian cultured cells (Yargeau et al., 2008).

Amoxicillin and the β -lactamase inhibitor potassium clavulanate formulation effluent was subjected to ozonation at varying pH (2.5–12.0) and ozone/hydrogen peroxide (perozonation) at differing initial hydrogen peroxide concentration by Alaton et al. (2004). The overall efficiency of COD removal varied between 10% and 56% for ozonation and 83% for the ozone/hydrogen peroxide process. The process at least partially removed the non-biodegradable COD fraction of the formulation effluent. The pretreatment of synthetic penicillin formulation effluent containing Procain Penicillin G resulted in more than 70% COD removal and a 50% decrease in acute toxicity towards *Daphnia magna* (Cokgor et al., 2006). The pretreatment process not only decreased the ultimate biodegradability of Procain Penicillin G effluent but also

increased its inhibitory effects on activated sludge treatment, possibly due to the formation of less biodegradable oxidation by-products. This example demonstrates that a thorough elucidation of the nature of degradation products is necessary.

4.5. Biodegradation

Most antibiotics tested to date have not been biodegradable under aerobic conditions (Richardson and Bowron, 1985; Al-Ahmad et al., 1999; Wiethan et al., 2000; Kümmerer et al., 2000; Ingerslev et al., 2001; Ingerslev and Halling-Sørensen, 2001; Thiele-Bruhn, 2003; Alexy et al., 2003, 2004; Gartiser et al., 2007a; Li et al., 2008c). Biodegradability has been poor for most of the compounds investigated in laboratory tests such as the OECD test series (301–303, 308) – even for some of the β -lactams (Alexy et al., 2004). Out of 16 antibiotics tested, only benzyl penicillin (penicillin G) was completely mineralized in a combination test (combination of the OECD 302 B and OECD 301 B tests; Gartiser et al., 2007a). Trials simulating sewage treatment (OECD 303 A) with radiolabeled compounds revealed that approximately 25% of benzyl penicillin was mineralized within 21 d, whereas ceftriaxone and trimethoprim were not mineralized at all (Junker et al., 2006). Carucci et al. (2006) found that the biodegradability of lincosamine in a sequence batch reactor was worse with municipal waste water than with synthetic waste water. No evidence of biodegradation for tetracycline was observed during a biodegradability test (sequence batch reactor), and sorption was found to be the principal removal mechanism for tetracycline in activated sludge (Kim et al., 2005).

Some antibiotics occurring in soil and sediment proved to be quite persistent in laboratory testing as well as in field studies. Some do not biodegrade well under anaerobic conditions (Gartiser et al., 2007b) others did (Maki et al., 2006). Substances extensively applied in fish farming had long half-lives in soil and sediment, as reported in several investigations (Jacobsen and Berglund, 1988; Hansen et al., 1992; Samuelsen et al., 1992, 1994; Hektoen et al., 1995; Capone et al., 1996; Marengo et al., 1997; Lai et al., 2008). However, some substances were at least partly degradable (Donoho, 1984; Gilbertson et al., 1990; Samuelsen et al., 1991, 1994; Capone et al., 1996; Thiele-Bruhn, 2003). Maki et al. (2006) found that ampicillin, doxycycline, oxytetracycline, and thiamphenicol were significantly degraded, while josamycin remained at initial levels. Tylosin was biodegraded (Hu and Coats, 2007).

5. Effects

If a substance is not eliminated in any way, it can reach the environment with the potential of adversely affecting aquatic and terrestrial organisms. It might reach humans again via drinking water. Until now, antibiotics have not yet been reported to be present in drinking water, only certain pharmaceuticals and diagnostics such as clofibrac acid or amidotrizoic acid have been reported to date. The effects of antibiotics on human health have been reported in the medical literature. Well known unwanted side effects within therapy are allergic reactions (e.g. β -lactams such as penicillin G or methicillin), and a few such as gentamicin are nephrotoxic. For quinolones it is known that sensitivity to light can be increased. Tetracyclines should not be applied for young children because of the negative interaction of tetracyclines with their developing teeth. Because of their antimicrobial activity, a negative interaction within the gut can happen within therapy. Bacteria, fungi and microalgae are the organisms primarily affected by antibiotics, because antibiotics are designed to affect microorganisms.

Antibiotics are of particular interest because we currently do not know whether their presence in natural waters contributes

to the spread of antibiotic resistance in microorganisms (Kümmerer, 2003). In general, the effects of antibacterial agents on bacteria and microalgae are found to be 2–3 orders of magnitude below the toxic values for higher trophic levels (Lanzky and Halling-Sørensen, 1997; Halling-Sørensen, 2000; Wollenberger et al., 2000; Brain et al., 2004, 2008; Robinson et al., 2005; Yamashita et al., 2006). This differential sensitivity is likely dependent on differences in metabolic potential as well as uptake kinetics (Brain et al., 2008), which has been demonstrated for a number of compounds from another class of biologically active compounds, namely, pesticides. Adverse impacts of antibiotics on higher aquatic organisms have been reported, but in most cases in which effects were detected the concentrations were environmentally irrelevant. However, secondary effects due to changes in the natural balance are not negligible.

For all tests involving bacteria one has to be aware that there are several shortcomings for the currently existing tests. One can either choose a pure culture, but in this case the test relies on specific toxicity. However, one cannot be sure that one is working with the most sensitive and/or most important organism. Furthermore, in the case of antibiotics most of them work preferentially against either Gram positive or Gram negative bacteria. This means that compounds active against Gram positive organisms will show no toxicity if a test with Gram negative bacteria is used such as the *Ps. putida* growth inhibition test, and vice versa. Alternatively, one can select a test that relies on a mixed population such as sewage sludge. In this case, effects against certain important organisms may be masked by other organisms that are either less or not at all affected but still contribute to the endpoint measured.

Acute tests seem to be inappropriate as a means of determining the effects of antibiotics on bacteria. Antibiotics possess specific modes of operation and impacts frequently become evident upon extending the incubation period. Toxicity tests with bacteria have shown that chronic exposure to antibiotics is critical rather than acute (Backhaus and Grimme, 1999, 2000; Froehner et al., 2000; Kümmerer et al., 2004). Thomulka and McGee (1993) determined the toxicity of a number of antibiotics (e.g. novobiocin, tetracycline, chloramphenicol, ampicillin, streptomycin) on *Vibrio harveyi* in two bioassay methods. Almost no toxic effects were found after short incubation times when luminescence was used as an endpoint. However, in a long term assay using reproduction as an endpoint a toxic effect in environmentally relevant concentrations could be detected for almost all the substances. These results are in accordance with the observations of Froehner et al. (2000). The same effect was found by Kümmerer et al. (2004) in tests with sewage sludge bacteria. A comparison of the results of short and long term bioassays with *Vibrio fischeri* demonstrate the risk of underestimating the severe effects of substances with delayed toxicity in acute tests. Similar findings concerning toxicity values were reported by Backhaus and Grimme (1999). In a long term bioluminescence inhibition test with *Vibrio fischeri*, toxic effect values (EC_{10}) were found for two antibiotics in the range of concentrations expected in the environment. Therefore, one should be aware that the results given for standard bacterial toxicity tests, such as short-term tests with the luminescent bacterium *Vibrio fischeri* and others, may underestimate effects and risks.

Effectiveness may be modulated by environmental conditions e.g. if bio-availability is reduced by sorption or activity is affected by complexation. If a substance is not eliminated in any way it can reach the environment with the potential to adversely affect the aquatic and terrestrial organisms.

Since experimental parameters influence the results of toxicity investigations, sometimes even by several orders of magnitude (Koller et al., 2000), the exact conditions of testing (e.g. temperature, pH value, time scale, etc.) have to be stated in order to be

able to assess impacts on the environment. Therefore, the effects outlined below should merely give an approximate indication of what may happen if an antibacterial is present in the environment. For a final conclusion knowledge about this issue is still too scarce.

5.1. Waste water and sewage system

Antibiotics have the potential to affect the microbial community in sewage systems. The inhibition of waste water bacteria may seriously affect organic matter degradation; therefore, effects of antibacterial agents on the microbial population are of great interest. A reduction in the number of bacteria together with alterations in microbial populations were observed in a model sewage purification system when different commonly applied antibiotics were added in concentrations that may occur in hospital waste water (Stanislawski, 1979; Al-Ahmad et al., 1999, in press; Kümmerer et al., 2000). As inhibitory concentrations in laboratory testing for a variety of antibiotics were found to be in the same order of magnitude as the concentrations expected for hospital waste water, the possibility of these substances affecting the microbial populations of hospitals' sewage systems could not be excluded.

Nitrification is an important step in waste water purification, eliminating toxic ammonia. The second step of nitrification, i.e. oxidation of nitrite to nitrate is particularly sensitive. Inhibition of this step under uncontrolled conditions may lead to accumulation of nitrite nitrogen in the plant effluent, a form of nitrogen which is particularly toxic. Several antibiotics proved to have low toxicity in relation to nitrifying bacteria in acute tests. These substances showed no effects upon nitrification in concentrations even higher than what might be environmentally expected (Tomlinson et al., 1966; Gomez et al., 1996). However, the time period of the test significantly influences the results (Halling-Sørensen, 2000; Kümmerer et al., 2004). An antimicrobial was found to require high concentrations in order to inhibit the nitrification process in a short term test (2–4 h), but a prolonged test period over 5 d showed effects one order of magnitude below the inhibitory concentrations of the acute test (Tomlinson et al., 1966). In a study by Dokianakis et al. (2004) the effects caused by the presence of seven different pharmaceuticals on a culture of nitrite-oxidizing bacteria isolated from activated sludge were reported. For ofloxacin and sulfamathoxazole significant inhibition was observed. In the same study, triclosan presented a substantial inhibitory effect on the substrate (nitrite) reduction rate. Lincomycin showed significant inhibition on nitrification activity in a SBR test, which is consistent with its antibiotic activity spectrum (Carucci et al., 2006). Christensen and co-workers found synergistic mixture effects of antibiotics against sewage sludge bacteria (Christensen et al., 2006).

Acetoclastic methanogenes are the most sensitive group of microorganisms participating in the anaerobic digestion process. Tests showed that the pharmaceuticals tested (among them sulfamathoxazole) caused mild inhibition of the methanogenes in most cases, which was in turn directly related to the tendency of the compounds to adsorb on the anaerobic biomass (Fountoulakis et al., 2004). In an ISO 13641 test antibiotics primarily active against Gram negative bacteria showed only moderate inhibition effects after a 7-d incubation period, with EC_{50} values between 24 mg L^{-1} and more than 1000 mg L^{-1} (equal to mg per g and day) (Gartiser et al., 2007b). In contrast, in the same study it was found that metronidazol which was optimized for activity against Gram positive bacteria was decisively toxic to anaerobic bacteria with an EC_{50} of 0.7 mg L^{-1} . In the anaerobic degradation tests according to ISO standard 11734 (1998), only benzyl penicillin showed definite final biodegradation after 60 d, while most antibiotics inhibited the digesting sludge in the respective parallel tested

inhibition controls. Thus, the inhibition of anaerobic bacteria by antibiotics observed in the degradation tests was higher than expected from the results of the inhibition tests. Possible explanations for this are that distinct substrates were used (yeast extract versus sodium benzoate), that the digestion sludge loses activity during the washing steps performed for the degradation tests and that the exposure time in the degradation tests was 8 times longer than in the inhibition test.

5.2. Surface water

Substances which are *not or are only* partly eliminated in the sewage treatment plant will reach surface water where they may affect organisms of different trophic levels. In a model aquatic system using synthetic fresh water, nitrifying bacteria were significantly affected by an aquaculture antibiotic. The disruption of the nitrification process already occurred in concentrations likely to be found in fish treatment tanks and sediments (Klaver and Matthews, 1994). The results of toxicity tests with bacteria indicate that adverse toxic effects on natural bacterial communities cannot be excluded.

The sensitivity of algae towards antibiotics varies widely. In an algal toxicity test *Selenastrum capricornutum* was found to be two to three orders of magnitude less sensitive to most antibiotics than microalgae *Microcystis aeruginosa*. The growth of *Microcystis aeruginosa* was inhibited at concentrations of less than 0.1 mg L^{-1} (Halling-Sørensen, 2000). Similar observations were documented by Holten-Lützhøft et al. (1999). Blue-green algae (cyanobacteria) seem to be sensitive to many antibiotics, for example amoxicillin, benzyl penicillin, sarafloxacin, spiramycin, tetracycline and tiamulin (Boxall et al., 2003). The potential ecotoxicological effect of the antibacterial substance metronidazol on *Chlorella* sp. and *Selenastrum capricornutum* in an acute toxicity test was outlined by Lanzky and Halling-Sørensen (1997). Their results indicated that potential adverse effects of antibiotics on algae could not be excluded. As algae are the basis of the food chain, even slight decreases in the algal population may affect the balance in an aquatic system.

Common receptors have been identified in plants for a number of antibiotics affecting chloroplast replication (fluoroquinolones), transcription and translation (tetracyclines macrolides, lincosamides, P-aminoglycosides, and pleuromutilins), metabolic pathways such as folate biosynthesis (sulphonamides) and fatty acid biosynthesis (triclosan) (Brain et al., 2008). Toxicological investigations into the potency of these compounds indicates susceptibility across multiple plant species, although sensitivity to these compounds varies widely between blue-green algae, green algae, and higher plants in a rather inconsistent manner, except that Cyanobacteria tend to be the most sensitive to antibiotic compounds (Brain et al., 2008). Effects on macrophytes have been investigated and the effect thresholds were found to be high (McGregor et al., 2007). Through single compound 7-d daily static renewal toxicity tests with *L. gibba*, sulfamathoxazole and levofloxacin were found to elicit phytotoxic effects in the concentration range utilized ($0\text{--}1000 \text{ } \mu\text{g L}^{-1}$) (Brain et al., 2004).

Exposure to antibiotics in the environment may have adverse reproductive effects in the early life stages of different organisms. This in turn may affect populations dramatically. A significantly depressed hatching rate for *Artemia* sp. cysts and a high mortality rate for *nauplii* as well as toxic effects on reproduction of *Daphnia magna* demonstrate how serious the impacts of antibiotics on these organisms are (Macrì et al., 1988; Migliore et al., 1993, 1997; Brambilla et al., 1994; Wollenberger et al., 2000). The capability of altering the pigmentation of *Artemia salina nauplii*, thus resulting in a loss of fitness for these individuals was demonstrated for the antibiotic flumequine. This underlines the toxic potential of

antimicrobial agents (Brambilla et al., 1994). LC_{50} values below 1 mg L^{-1} for the antibacterial agent furazolidone demonstrated toxicity on *Culex pipiens molestus* larvae, *Daphnia magna* and *Artemia salina* (Macrì et al., 1988). Based on these results, the authors outlined the possibility of considerable damage to the natural equilibrium since the organisms under investigation constitute the nourishment for other aquatic animals, and therefore their disappearance affects other organisms as well. Beside the impacts on the populations outlined above, antibiotics in the environment can also affect the behavior of aquatic organisms. For instance, it has been shown that antibiotics can influence the phototaxis of *Daphnia magna* (Dojmi di Delupis et al., 1992; Brambilla et al., 1994).

Antimicrobial agents are not likely to affect fish adversely. In each of the studies, effects were either found only at high, environmentally unrealistic concentrations, or no toxic effects are observed at all. Toxicity tests using different fish species (*Acartia tonsa*, *Brachydanio rerio*, *Leup totes reticulatus*, *Salmo gairdneri*, *Salvelinus namayeuish*) showed no antibiotic toxicity against the species tested (Canton and van Esch, 1976; Marking et al., 1988; Lanzky and Halling-Sørensen, 1997). An antimicrobial extensively applied in aquaculture has been reported to cause skeletal deformations; however, the substance is applied at concentrations higher than those expected to be normally found in the environment (Lunestad, 1992).

In a study by Kim et al. (2007) sulfamathoxazole, sulphachlorpyridazine, sulfathiazole, sulphamethazine, sulphadimethoxine, and trimethoprim were examined for their acute aquatic toxicity by employing a marine bacterium (*Vibrio fischeri*), a freshwater invertebrate (*Daphnia magna*), and the Japanese medaka fish (*Oryzias latipes*). In this study *Daphnia* was in general the most susceptible among the test organisms. Predicted environmental concentrations (PECs) derived for the test pharmaceuticals in Korea ranged between 0.14 and $16.5 \text{ } \mu\text{g L}^{-1}$. The hazard quotients derived from PECs and the predicted no effect concentrations (PNEC) for sulfamathoxazole were 6.3 and 1.8, respectively, for Korea suggesting potential environmental concerns and a need for further investigation. In another study (Park and Choi, 2008) eleven commonly used antibiotics including sulphonamides, tetracyclines, aminoglycosides, fluoroquinolones, and β -lactams were evaluated for their acute and chronic aquatic toxicities using standard test organisms e.g. *Vibrio fischeri*, *Daphnia magna*, *Moina macrocopa*, and *Oryzias latipes*. Among the antibiotics tested for acute toxicity, neomycin was the most toxic followed by trimethoprim, sulfamathoxazole and enrofloxacin. Sulphamethazine, oxytetracycline, chlortetracycline, sulphadimethoxine and sulfathiazole were of intermediate toxicity, while ampicillin and amoxicillin were the least toxic for the test organisms. There were no trends in sensitivity among test organisms or among the different classes of antibiotics. Only the β -lactam class was the least toxic. In the chronic toxicity test, neomycin affected reproduction and adult survival of *D. magna* and *M. macrocopa* with low mg L^{-1} levels exposure. The lower effects of the β -lactams could be due to their susceptibility to hydrolysis (see above). This example suggests the importance of monitoring concentrations during toxicity testing.

Ciprofloxacin was active against *Vibrio fischeri* at a high concentration (5 mg L^{-1}) (Hernando et al., 2007). The toxic effects of the antibacterial agents levofloxacin and clarithromycin on aquatic organisms was studied by Yamashita et al. (2006). Microtox tests with *Vibrio fischeri* showed that the compounds studied have no acute toxicity for the bacterium. Toxicity tests with bacteria have shown that chronic exposure to antibiotics is critical rather than acute (see above). No effects were found by Yamashita et al. (2006) in the *Daphnia* immobilisation test. However, in an algal growth inhibition test the authors found that levofloxacin and clarithromycin are both highly toxic for the microalgae. The phyto

toxicity of clarithromycin was about 100-fold higher than that of levofloxacin (EC_{50}). In the *Daphnia* reproduction test, levofloxacin and clarithromycin also showed chronic toxicity against the crustacean. Based on the PEC/PNEC ratio the authors considered the ecological risk of levofloxacin to be low, however that of clarithromycin is higher, suggesting that clarithromycin may affect organisms in the aquatic environment.

Robinson et al. (2005) performed toxicity tests with seven fluoroquinolone antibiotics (ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, clinafloxacin, enrofloxacin) and flumequine, on five aquatic organisms. Overall toxicity values ranged from 7.9 to 23 000 $\mu\text{g L}^{-1}$. The cyanobacterium *Microcystis aeruginosa* was the most sensitive organism (5-d growth and reproduction, effective concentrations [EC_{50} s] ranging from 7.9 to 1960 $\mu\text{g L}^{-1}$ and a median of 49 $\mu\text{g L}^{-1}$), followed by duckweed (*Lemna minor*, 7-d reproduction, EC_{50} values ranged from 53 to 2470 $\mu\text{g L}^{-1}$ with a median of 106 $\mu\text{g L}^{-1}$) and the green alga *Pseudokirchneriella subcapitata* (3-d growth and reproduction, EC_{50} values ranged from 1100 to 22 700 $\mu\text{g L}^{-1}$ with a median 7400 $\mu\text{g L}^{-1}$). Results from tests with the crustacean *Daphnia magna* (48-h survival) and fathead minnow (*Pimephales promelas*, 7-d early life stage survival and growth) showed limited toxicity with no-observed-effect concentrations at or near 10 mg L^{-1} . At an estimated environmental concentration of 1 $\mu\text{g L}^{-1}$ only *M. aeruginosa* may be at risk in surface water. However, the authors hypothesized that selective toxicity of these compounds may have implications for aquatic community structure.

5.3. Sediments

Several papers have addressed the impacts of antibiotics on soil dwelling organisms. Antimicrobials may have qualitative and quantitative effects upon the resident microbial community found in sediment, which can in turn affect the degradation of organic matter. Furthermore, direct toxic effects upon the resident organisms cannot be excluded (Nygaard et al., 1992; Kong et al., 2006). The situation in sediments beneath fish farms is critical because of the high local antimicrobial concentrations. Some antimicrobial agents have been seen to reduce the number of bacteria at concentrations which are relevant to fish farm sediments. Organism activity is also affected. A temporary effect on sulphate reduction was observed when antibiotics were added to sediment, either due to the growth of sulphate reducing bacteria, or of the fermenting and acetogenic bacteria supplying them with substrate being inhibited (Hansen et al., 1992). Antibiotics present in sediment can lose their antimicrobial activity as a result of their binding to sediment particles or their forming complexes with ions. This ability has already been demonstrated for some substances. However, contradictory results concerning the loss of antibacterial activity due to binding or complex formation have been found for one and the same substance (Lunestad and Goksøyr, 1990; Björklund et al., 1991; Hansen et al., 1992; Hektoen et al., 1995, see also above subchapter on sorption). The reason for these contradictory results could be due to differences in sediment composition, which seem to play a key role in the effects of substances upon the resident population, because the composition of the sediment or soil determines the degree and strength of sorption. The magnitude of the effect of ciprofloxacin on microbial salt marsh communities, for example, was inversely correlated to the degree of sorption to the sediments. Despite the fact that ciprofloxacin is a broad-spectrum antibiotic, its impact on sediment microbial communities was selective and appeared to favor sulfate-reducing bacteria and Gram negative bacteria (Córdova-Kreylos and Scow, 2007). The potential of a pollutant to accumulate in organisms has to be considered critical. Antibiotics which are poorly water soluble, especially if the bioconcentration factor is between 500 and 1000 or the oct-

anol/water distribution coefficient exceeds the value of 1000, tend to accumulate in organisms. The enrichment of substances in organisms has been shown for some antibiotics, e.g. sulphadimethoxine (Lunestad, 1992; Migliore et al., 1993).

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