Environmental Pollution xxx (2017) 1–11

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Antimicrobial activity of pharmaceutical cocktails in sewage treatment plant effluent – An experimental and predictive approach to mixture risk assessment☆

Jakob Menz [a], Ewelina Baginska [a], Åsa Arrhenius [b], Annette Haiß [a], Thomas Backhaus [b], Klaus Kümmerer [a, *]

[a] Sustainable Chemistry and Material Resources, Institute of Sustainable and Environmental Chemistry, Leuphana University Lüneburg, Scharnhorststrasse 1, DE-21335 Lüneburg, Germany
[b] Department of Biological and Environmental Sciences, University of Gothenburg, Carl Skottsbergs Gata 22B, Box 461, 40530 Gothenburg, Sweden

ARTICLE INFO

Article history:
Received 22 April 2017
Received in revised form
31 August 2017
Accepted 5 September 2017
Available online xxx

Keywords:
Antibiotics
Mixture toxicity
Environmental bacteria
Microbial communities

ABSTRACT

Municipal wastewater contains multi-component mixtures of active pharmaceutical ingredients (APIs). This could shape microbial communities in sewage treatment plants (STPs) and the effluent-receiving ecosystems. In this paper we assess the risk of antimicrobial effects in STPs and the aquatic environment for a mixture of 18 APIs that was previously detected in the effluent of a European municipal STP. Effects on microbial consortia (collected from a separate STP) were determined using respirometry, enumeration of culturable microorganisms and community-level physiological profiling. The mixture toxicity against selected bacteria was assessed using assays with *Pseudomonas putida* and *Vibrio fischeri*. Additional data on the toxicity to environmental bacteria were compiled from literature in order to assess the individual and expected joint bacterial toxicity of the pharmaceuticals in the mixture. The reported effluent concentration of the mixture was 15.4 nmol/l and the lowest experimentally obtained effect concentrations (EC_{10}) were 242 nmol/l for microbial consortia in STPs, 225 nmol/l for *P. putida* and 73 nmol/l for *V. fischeri*. The lowest published effect concentrations (EC_{50}) of the individual antibiotics in the mixture range between 15 and 150 nmol/l, whereas 0.9–190 μmol/l was the range of bacterial EC_{50} values found for the non-antibiotic mixture components. Pharmaceutical cocktails could shape microbial communities at concentrations relevant to STPs and the effluent receiving aquatic environment. The risk of antimicrobial mixture effects was completely dominated by the presence of antibiotics, whereas other pharmaceutical classes contributed only negligibly to the mixture toxicity. The joint bacterial toxicity can be accurately predicted from the individual toxicity of the mixture components, provided that standardized data on representative bacterial strains becomes available for all relevant compounds. These findings argue for a more sophisticated bacterial toxicity assessment of environmentally relevant pharmaceuticals, especially for those with a mode of action that is known to specifically affect prokaryotic microorganisms.

1. Introduction

Most active pharmaceutical ingredients (APIs) undergo only incomplete resorption and metabolism within the human body and therefore enter the raw sewage as active compounds or as metabolites (Jelić et al., 2012). Furthermore, parent APIs can enter raw sewage via the inappropriate disposal of out-of-date or unwanted drugs (Kümmerer, 2008). These pharmaceutical residues form multi-component mixtures in municipal wastewater that are only insufficiently removed during the passage through the sewage treatment plant (STP) (Fatta-Kassinos et al., 2011; Heberer, 2002; Roberts and Thomas, 2006). The ecotoxicological risk of pharmaceutical mixtures typically exceeds the risk of each individual compound (Backhaus, 2016). Consequently, there is an urgent need for ecotoxicity assessment of pharmaceutical mixtures in environmentally realistic settings, i.e. scenarios where several interacting

☆ This paper has been recommended for acceptance by Charles Wong.

* Corresponding author.
E-mail address: klaus.kuemmerer@leuphana.de (K. Kümmerer).

https://doi.org/10.1016/j.envpol.2017.09.009
0269-7491/

species are exposed to a multitude of pharmaceuticals from different classes (Backhaus, 2014; Vasquez et al., 2014).

The occurrence of APIs in the environment impacts microbial communities in different ways (Barra Caracciolo et al., 2015). Microbial communities can acquire tolerance in polluted environments as a result of an adaptation or acclimatization of populations or from shifts in species composition due to altered competitive interactions under toxic exposure (Tlili et al., 2015). The ability of complex microbial communities to adapt to the presence of multiple toxic compounds is important for the process stability in biological wastewater treatment systems (Kong et al., 1993). It was found that the chemical, rather than the bacterial composition of the incoming wastewater represents the main factor in shaping the microbial community structure in activated sludge (Shchegolkova et al., 2016). Therefore, it is not surprising that mixtures of APIs from different classes were shown to potentially influence activated sludge communities (Kraigher et al., 2008; Wang and Gunsch, 2011). In this context, antibiotic agents are of particular interest, since they possess a high intrinsic potential to affect the microbial degradation of organic matter in STPs (Kümmerer, 2009). Moreover, their presence in STPs at sub-inhibitory concentrations may promote the selection and spread of antibiotic resistance (Rizzo et al., 2013). There are many indications that APIs, either in mixtures or even as single compounds, may shape not only microbial communities in STPs, but also sensitive natural microbial communities in the aquatic environment (Corcoll et al., 2014; Ebert et al., 2011; Lawrence et al., 2005; Veach et al., 2012; Wilson et al., 2003). Such effects may have far-reaching consequences, since alterations of the natural microbial composition could impact ecosystem functioning (Reed and Martiny, 2007). Moreover, antibiotics can exert a selective pressure at concentrations up to several hundred-fold below the minimal inhibitory concentration of susceptible bacteria (Gullberg et al., 2011) and reported antibiotic concentrations in STP effluent often exceeded predicted no effect concentrations for resistance selection (Bengtsson-Palme and Larsson, 2016; Kümmerer and Henninger, 2003). As a consequence, the combined discharge of antibiotics and antibiotic-resistant bacteria from STPs may contribute to the maintenance and spread of antibiotic resistance in the aquatic environment (Goñi-Urriza et al., 2000).

The current guideline of the European Medicines Agency (EMA) for the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr 2) does not require the mandatory evaluation of antimicrobial effects for all pharmaceuticals prior to marketing authorization (EMA, 2006). The first step (phase I) of the tiered assessment procedure estimates the exposure of the environment to the drug substance. If phase I results in a predicted environmental concentration (PEC) for the aquatic compartment equal to or above 0.01 μg/l, a screening-level assessment of aquatic fate and effects should be conducted in Tier A of phase 2. The Tier A assessment of antimicrobial effects, i.e. the inhibition of activated sludge respiration and cyanobacteria growth, is only mandatory for pharmaceuticals used for antimicrobial purposes. If a risk for effects on microorganisms cannot be excluded in Tier A, additional testing on specific bacteria species (e.g. *Pseudomonas putida*) is required as part of Tier B of phase II.

The importance of making more use of ecotoxicological endpoints targeting microorganisms and microbial communities in environmental risk assessment of antibiotics was recently pointed out (Brandt et al., 2015). Besides, the EMA guideline does not address the effects of mixtures that are formed unintentionally in sewage and the aquatic environment. The risk assessment of pharmaceutical cocktails represents a challenging task since environmental mixtures are highly variable in terms of composition and concentration (Vasquez et al., 2014). Moreover, the high demands on data availability still impede the accurate modeling of cocktail effects (Backhaus, 2016). However, a tiered approach for the predictive screening-level risk assessment of chemical mixtures on the basis of the Concentration Addition (CA) concept was introduced in 2012 (Backhaus and Faust, 2012) and the first tier of this approach was applied to multi-component pharmaceutical mixtures that reportedly occurred in European STP effluents (Backhaus and Karlsson, 2014). This resulted in the indication of substantial risks that were mainly attributed to the specific toxicity of antibiotic agents against cyanobacteria. Hence, pharmaceutical mixtures in the effluent of municipal STPs may pose a particular risk to prokaryotic microorganisms.

This study seeks to further characterize the risk of antimicrobial effects that was previously indicated for a pharmaceutical mixture that was found in the effluent of a STP in Europe (Andreozzi et al., 2003; Backhaus and Karlsson, 2014). For this purpose, a synthetic multi-component mixture of 18 APIs was designed according to the effluent monitoring data that was originally reported by Andreozzi et al. (2003). This mixture was tested for its toxicity to microbial communities in STPs and to selected bacterial species using a multiple endpoint approach. In addition, available data on the toxicity to environmental bacteria were compiled from peer-reviewed literature in order to determine the individual and the estimated joint bacterial toxicity of the individual mixture components.

2. Material and methods

2.1. Preparation of the pharmaceutical mixture

A master solution containing 18 different APIs was prepared on the basis of measured effluent concentrations (MECs) that were previously reported for a European STP (M1-I: Latina, Italy) (Andreozzi et al., 2003). Stock solutions of the 18 individual pharmaceuticals were prepared in methanol and stored at −18 °C until further use. Aliquots of the stock solutions were combined, evaporated under a gentle stream of air until dryness and redissolved in 500 ml ultrapure water to produce a 40,000 fold concentrate of the MEC. The reported MEC and the nominal concentration in the master solution of each component are presented in Table 1. The total measured concentration of the mixture, i.e. the sum of the individual MECs, was 15.4 nmol/l.

2.2. Testing of effects on microbial communities from STPs

The specific characteristics of the investigated exposure scenario necessitated some adaptations of the standard information requirements, i.e. the respiration inhibition test according to OECD TG 209, which can be justified as follows.

i) The conventional respiration inhibition test (OECD 209) fails to assess the effects of antibiotics because of the short test duration (Kümmerer et al., 2004). Therefore, a prolonged test was conducted in analogy to OECD guideline 301 F, which is a continuous respirometric method that was originally developed for the assessment of ready biodegradability (OECD, 1992). The toxicity control of OECD TG 301 F can be considered reliable for observing inhibitory effects of the testing material (European Commission, 2003; ECHA, 2016).

ii) Concentrations of the investigated pharmaceuticals in the influent and within the STP were not available. Therefore, the toxicity of the mixture found in the effluent was determined by using STP effluent as inoculum instead of activated sludge. The bioavailable concentration in the aeration tank, i.e. the dissolved concentration to which the microorganisms are exposed, can be expected to be approximately equal to the effluent concentration

Please cite this article in press as: Menz, J., et al., Antimicrobial activity of pharmaceutical cocktails in sewage treatment plant effluent – An experimental and predictive approach to mixture risk assessment, Environmental Pollution (2017), https://doi.org/10.1016/j.envpol.2017.09.009